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Immunizing against polio

Polio has re-emerged in some countries in the Region that had previously been polio-free for years, emphasising the urgent need to ensure vaccination of all children.

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المجلة الصحية لشرق المتوسط

هي المجلة الرسمية التي تصدر عن المكتب الإقليمي لشرق المتوسط بمنظمة الصحة العالمية. وهي منبر لتقديم السياسات والمبادرات الجديدة في الخدمات الصحية والترويج لها، ولتبادل الآراء والمفاهيم والمعطيات الوبائية ونتائج الأبحاث وغير ذلك من المعلومات، وخاصة ما يتعلق منها بإقليم شرق المتوسط. وهي موجهة إلى كل أعضاء المهن الصحية، والكليات الطبية وسائر المعاهد التعليمية، وكذا المنظمات غير الحكومية المعنية، والمراكز المتعاونة مع منظمة الصحة العالمية والأفراد المهتمين بالصحة في الإقليم وخارجه.

EASTERN MEDITERRANEAN HEALTH JOURNAL

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LA REVUE DE SANTÉ DE LA MÉDITERRANÉE ORIENTALE

EST une revue de santé officielle publiée par le Bureau régional de l'Organisation mondiale de la Santé pour la Méditerranée orientale. Elle offre une tribune pour la présentation et la promotion de nouvelles politiques et initiatives dans le domaine des services de santé ainsi qu'à l'échange d'idées, de concepts, de données épidémiologiques, de résultats de recherches et d'autres informations, se rapportant plus particulièrement à la Région de la Méditerranée orientale. Elle s'adresse à tous les professionnels de la santé, aux membres des instituts médicaux et autres instituts de formation médico-sanitaire, aux ONG, Centres collaborateurs de l'OMS et personnes concernés au sein et hors de la Région.

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Editorial

An ancient scourge triggers a modern emergency

Bruce Aylward¹

The Eastern Mediterranean – crossroads of the world. Sitting for millennia on ancient, vital trade and travel routes, leaders here have faced a twofold challenge in protecting their people from the ravages of infectious diseases: first, the control of pathogens native to these lands and, secondly, the elimination of those pathogens which gain entry with travellers, traders and pilgrims. The most dangerous of this latter group are those organisms that enter the region silently, then spread widely before suddenly emerging with terrible consequences for the most vulnerable populations.

This past month, an ancient virus again re-emerged in the Middle East, more than a decade after most health leaders thought it had been vanquished there forever.

On 28 October 2013, the Minister of Health of the Syrian Arab Republic announced to his counterparts from 22 countries of the Eastern Mediterranean Region of the World Health Organization, that after a 15-year absence, polio was again paralysing and killing children in his country. Genetic sequencing showed that the virus had originated in Pakistan and already travelled to Egypt, Israel, the Gaza Strip and the West Bank over the previous 12 months.

Within 24 hours, the assembled Ministers declared this re-infection of the Middle East an emergency for the entire Eastern Mediterranean Region, calling for extraordinary joint action to combat this ancient scourge [1]. The Minister of Oman announced US\$ 5 million in new financing for the effort, Saudi Arabia declared it would mobilize

religious leaders to ensure that all parents understood their obligation to vaccinate their children, the United Arab Emirates reconfirmed their US\$ 120 million pledge for polio made earlier this year; most striking, 7 countries immediately agreed to coordinate the vaccination of 22 million children over the subsequent 3 weeks, and again in December, to again vanquish polio in the Middle East. All countries called on Pakistan to rapidly access and vaccinate all of its children as a matter of urgency to stem the international spread of its viruses.

This decisive leadership and rapid emergency action builds on a long and illustrious history of infectious disease control, vaccination and, especially, polio eradication in the Eastern Mediterranean Region.

A few examples reinforce the striking accomplishments of this Region on its road to becoming polio-free.

When poliovirus roared out of northern Nigeria 10 years ago following the temporary suspension of oral poliovirus vaccine (OPV) in 2 states, nearly 2 dozen previously polio-free countries became re-infected and thousands of children were needlessly paralysed; Saudi Arabia led the world in boldly introducing new polio vaccination requirements for all travellers from polio-infected countries to protect pilgrims, travellers and Saudi Arabians alike [2]. When it appeared impossible to interrupt polioviruses in Egypt due to the very high population density, particularly in the mega-city of Cairo/Giza, that country led the world in

innovation by commissioning the fast-track development and use of a new monovalent OPV (mOPV1); polio transmission there stopped almost immediately. In Afghanistan, the Ministry of Public Health courageously supported the painstaking work of negotiating vaccinator access to every corner of Kandahar and Helmand provinces; as of November 2013, the country passes its first anniversary with no child having been paralysed by an indigenous poliovirus^a.

As importantly, major regional institutions – including the Organization of Islamic Cooperation and the Islamic Development Bank – have brought their voices and resources to the effort to secure a polio-free world. Religious leaders, led by the Grand Imam of Al Azhar, have formed an Islamic Advisory Group for the Global Polio Eradication Initiative to ensure parents know that they are obligated to ensure all children are vaccinated and to ensure communities assure the safe passage and work of vaccinators.

This central role of the Eastern Mediterranean in polio eradication led global leaders in philanthropy, development and vaccinology to gather in Abu Dhabi in April 2013 to launch the new *Polio Eradication & Endgame Strategic Plan 2013–2018* [3] and pay tribute to the commitment of the Region's leaders to immunization and disease eradication.

The major unresolved threat to the Region's deep commitment to complete polio eradication is now the decision by a handful of local leaders in

^aFor the first time in history, all polio cases detected in Afghanistan in 2013 have been due to imported viruses (originating in Pakistan's Federally Administered Tribal Areas or FATA).

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parts of north-west Pakistan and south/central Somalia to withhold vaccination and protection from the devastating effects of this disease. As national and regional leaders launch their new emergency eradication effort to again eliminate polio in the Middle East, they must at the same time rapidly reconcile the concerns of local leaders in Pakistan

and Somalia to restart vaccination in those areas.

2014 must be the year in which the Eastern Mediterranean conquers this ancient menace by backing this emergency response with the leadership, generosity, innovation, determination and diligence that has been characteristic of this Region's work in polio eradication

and which continues to inspire the entire Global Polio Eradication Initiative.

The first recorded image of polio in the world is from the Eastern Mediterranean, where the consequences of this disease were captured in a 5000 year-old stele from Egypt. There is absolutely no reason why the last image of polio should be from this Region.

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Arabic version of the Global Mental Health Assessment Tool—Primary Care version (GMHAT/PC): a validity and feasibility study

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النسخة العربية من الأداة العالمية لتقييم الصحة النفسية - نسخة الرعاية الأولية: دراسة حول صحتها وإمكانية استخدامها
فيال شارما، شازيا دوراني، ماثيو سوا، جون كوبلاند، محمد أبو صالح، ستيفن لين، بيتر لينغ

الخلاصة: يشوب النقص خدمات الصحة النفسية في إقليم شرق المتوسط. ولما كانت الأداة العالمية لتقييم الصحة النفسية - نسخة الرعاية الأولية هي أداة محوسبة ومهيكلية جزئياً للتقييم السريري، أعدت كي تساعد العاملين الصحيين على إجراء تقييمات سريعة وملائمة وشاملة ومعيارية؛ فقد أجرى الباحثون هذه الدراسة في الإمارات العربية المتحدة لتقييم جدوى وإمكانية استخدام النسخة العربية لهذه الأداة. وقد طبقت ممرضات الصحة النفسية هذه الاستمارة على خمسين مريضاً في مواقع الصحة النفسية والتأهيل النفسي؛ ثم قارن الباحثون التشخيص لديهم بالتشخيص السريري في الطب النفسي المستقل الذي يستند على الطبعة العاشرة من التصنيف الدولي للأمراض للمرضى ذاتهم. ووجدت الممرضات أن الأداة أكثر سهولة في التطبيق، وتستغرق 16 دقيقة، وكان هناك توافق جيد بين التشخيص المستند على الأداة وبين تشخيص الأطباء النفسيين (كبا = 0.91)، وكانت الحساسية مرتفعة (97%)، والنوعية مرتفعة (94%).

ABSTRACT Mental health services are far from satisfactory in the Eastern Mediterranean Region. The Global Mental Health Assessment Tool—Primary Care version (GMHAT/PC) is a semi-structured, computerized clinical assessment tool that was developed to assist health workers in making quick, convenient and comprehensive standardized mental health assessments. A study was carried out in the United Arab Emirates to evaluate the validity and feasibility of the Arabic version of the GMHAT/PC. Mental health nurses administered the GMHAT/PC Arabic version to 50 patients in mental health and rehabilitation settings and their GMHAT/PC diagnosis was compared with the psychiatrist's independent ICD-10 based clinical diagnosis on the same patients. The nurses found GMHAT/PC easy to administer in an average of 16 minutes. The GMHAT/PC-based diagnosis had a good agreement with the psychiatrist's diagnosis (kappa = 0.91) and a high sensitivity (97%) and specificity (94%).

Version en langue arabe de l'outil d'évaluation mondial de la santé mentale dans le monde - soins primaires : étude de validité et de faisabilité

RÉSUMÉ Les services de santé mentale sont loin d'être satisfaisants dans la Région de la Méditerranée orientale. L'outil d'évaluation mondial de la santé mentale - version pour les soins primaires - est un instrument d'évaluation clinique semi-structuré assisté par ordinateur qui a été élaboré pour permettre aux agents de santé d'établir rapidement et facilement des évaluations de santé mentale standardisées et exhaustives. Une étude a été menée aux Émirats arabes unis afin d'évaluer la validité et la faisabilité de la version en langue arabe de cet outil d'évaluation. Des infirmières en santé mentale ont utilisé la version en langue arabe de cet outil d'évaluation sur 50 patients en milieu de psychiatrie et de réadaptation. Les diagnostics issus de l'évaluation ont été comparés aux diagnostics cliniques établis à l'aide de la CIM-10 par des psychiatres indépendants pour les mêmes patients. Les infirmières ont trouvé que l'outil d'évaluation mondiale de la santé mentale - version pour les soins primaires - était facile à administrer ; la tâche prenait 16 minutes en moyenne. Le diagnostic établi à l'aide de cet outil d'évaluation avait un degré de concordance satisfaisant avec le diagnostic du psychiatre (kappa = 0,91) et avait une sensibilité (97 %) et une spécificité (94 %) élevées.

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Introduction

Mental health services are far from satisfactory in the Eastern Mediterranean Region (EMR). The World Health Organization's (WHO) recent report [1] highlighted limited resources available for the care of people with mental illness, poor utilization of these resources and as a consequence a significant treatment gap of up to 85%. The report concluded that mental health resources were scarce, inequitably distributed and inefficiently used; community-based mental health services were underdeveloped; and collaboration between the mental health system and other health and non-health sectors was generally weak in the Region.

Similar to more developed countries, between 20% and 34% of consultations with primary health care facilities in the EMR are due to mental health problems [2]. Health officials need to understand and appreciate the harmful effects of mental illness and give attention to prevention, treatment and rehabilitation of mental disorders at the primary care level as well as other levels of care [3]. It is high time to reduce the gap between the needs and the services offered. To address the issue of low detection rates of psychiatric disorders in Arab cultures, screening instruments have been translated and validated into Arabic language: the General Health Questionnaire and the Self-Reporting Questionnaire [4] and new screening instruments have been developed [5] including a culture-oriented screening scale for anxiety and depression [6].

One pragmatic way to reduce the treatment gap for mental illness in the EMR and other parts of the world is by providing front-line workers with the skills to recognize and manage common mental illness and to identify patients with severe illness at their earlier stage so that they can be helped through specialist services. Based on their extensive clinical and research

experience Sharma, Copeland and others have spent over 15 years developing the Global Mental Health Assessment Tool—Primary Care version (GMHAT/PC), a computer-assisted clinical tool to assess and diagnose and treat mental illness in primary and general health care settings. This has been further refined by trials in routine clinical practice in different settings, with input from general practitioners, patients and carers. GMHAT/PC was subjected to reliability and validity studies in primary care as well as in medical settings including among older people [7–11]. The aim of the present study was to validate the Arabic version of the GMHAT/PC and to examine its feasibility and acceptability in an Arab population.

Methods

Description of the GMHAT/PC

The GMHAT/PC is a semi-structured, computerized clinical assessment tool that is developed to assist health workers in making quick, convenient and comprehensive standardized mental health assessments in both primary and general health care.

The program starts with basic instructions giving details of how to use the assessment tool and rate the symptoms. The first 2 screens help in getting brief background details including present, past, personal and social history including history of trauma, epilepsy and learning disorders. The following screens consist of a series of questions leading to a comprehensive yet quick mental state assessment. They start with 2 screening questions about every major symptom complex followed by additional questions only if the screening questions are positive. The questions cover the following symptom areas: worries, anxiety and panic attacks, concentration, depressed mood, including suicidal risk, sleep, appetite, eating disorders, hypochondriasis, obsessions

and compulsions, phobia, mania, psychotic symptoms, disorientation, memory impairment, alcohol misuse, illegal drug misuse, personality problems and stressors. The questions proceed in a clinical order along a tree-branch structure. Many of the GMHAT/PC items have been adapted for the full adult range from the Geriatric Mental State (GMS/AGECAT) schedule [12], which is extensively used worldwide in numerous epidemiological studies. Ratings are made by the interviewer using his or her clinical skills to judge the severity of each symptom, thus making the GMHAT/PC a semi-structured interview.

The computer-assisted diagnostic algorithm takes account of clinical diagnostic practices based on presence of symptoms. The printable output summary report includes background descriptive details, a list of symptoms with their severity as well as their scores, risk of self-harm, the GMHAT/PC main diagnosis and additional diagnoses. The additional diagnoses or comorbid states, are based on the presence of other mental illness symptoms and disorders. Clinicians who used GMHAT/PC found the list of all possible mental health diagnoses very useful, as it helped them in their overall understanding of the patients' mental health issues and for planning their treatments.

The program contains evidence-based management guidelines for most disorders, and for most psychotic disorders recommends referral to mental health services. If interviews are repeated over time on a patient, the program also produces a summary table of symptom ratings of all interviews, providing a clear indication of progress between interviews

Study design

The Arabic version of GMHAT/PC was developed using the standard method used in the translation of GMHAT/PC into other languages. The GMHAT/

PC questions were translated into standard Arabic language by a clinician with a sound knowledge of Arabic. An independent translator translated back from Arabic to English. The English back translation was compared with the original GMHAT/PC questions by the GMHAT/PC steering group. The Arabic version was used in this study for interviews. We also assessed whether the tool was acceptable in this culture.

Data collection

The study was carried out in 2 settings in Abu Dhabi, as follows.

Mental health setting

One trained psychiatric nurse used GMHAT/PC for assessment of all patients attending the outpatient clinic in the Behaviour Science Pavilion, Abu Dhabi, United Arab Emirates. Patients were informed and included after obtaining their consent to take part in the study. One qualified psychiatrist made clinical assessment independently and arrived at clinical diagnosis based on the *International Statistical Classification of Diseases and Related Health Problems*, 10th revision (ICD-10). The psychiatrist was unaware of the computer (GMHAT/PC) diagnosis and of any previous mental health problems. Patients were referred to this clinic by general practitioners, liaison psychiatry teams or other health teams, and had varying degrees of mental illness.

Rehabilitation setting

In the rehabilitation unit another community nurse used GMHAT/PC and the psychiatrist attached to the unit made an independent clinical assessment, as described above. Most of the patients included had a history of mental health problems. The rehabilitation setting included a day centre and community care mental health team. Patients were referred to this service from outpatients, inpatients to help early discharge and home care teams.

Results

A total of 50 patients were interviewed (23 men and 27 women). The age range was 19–69 years, with a mean age of 37 years. The mean time taken for the interviews was 16 minutes. None of the patients declined to be interviewed.

A total of 17 patients were identified by the psychiatrist as having no mental health illness, while 33 patients were identified as having mental health illness. There were no significant differences in ages between the 2 groups: mean ages were 38.4 and 36.2 years respectively. Similarly there was also no significant difference in the sex distribution between the 2 groups.

Of 33 patients diagnosed as having mental illnesses by the psychiatrist 32 were also diagnostic cases of mental illness according to nurses administering

the GMHAT/PC. Of the 17 cases without mental illness diagnosis by the psychiatrist, 16 were correctly diagnosed as having no illness by GMHAT/PC, thus giving a kappa value for diagnostic agreement of 0.91 (95% CI: 0.79–1.00) with sensitivity of 97% (95% CI: 91%–100%) and specificity of 94% (95% CI: 83%–100%).

For anxiety and depression the kappa value for diagnostic agreement was 0.75 (95% CI: 0.56–0.96), with a sensitivity of 86% (12/14) (95% CI: 67%–100%) and a specificity of 92% (33/36) (95% CI: 83%–100%). For psychosis, the diagnostic agreement kappa value was 0.76 (95% CI: 0.52–0.96), with a sensitivity of 71% (10/14) (95% CI: 47%–95%) and specificity of 97% (35/36) (95% CI: 91%–100%).

The cross-tabulation of psychiatrist's ICD-10 based clinical diagnoses and GMHAT/PC diagnoses is given in Table 1.

Finally, basic feedback was also obtained from the patients and interviewers. All patients were asked how they felt about the interview and whether they understood the questions. The nurses who administered GMHAT/PC were asked about their feedback on using the tool. The patients easily understood the questions and readily accepted the interview. The feedback from the nurses' interviews was generally very positive,

Table 1 Cross-tabulation of the number of patients diagnosed by the psychiatrist based on clinical judgement and by the nurse using the Global Mental Health Assessment Tool—Primary Care version (GMHAT/PC)

Psychiatrist clinical diagnosis	Nurse GMHAT/PC diagnosis						Total
	No mental illness	Organic mental disorder	Psychosis	Depression	Anxiety/neurosis	Eating disorder	
No mental illness	16	0	0	0	0	1	17
Organic mental disorder	0	1	0	0	0	0	1
Psychosis	0	3	10	1	0		14
Depression	0	0		9	0	1	10
Anxiety/neurosis	1	0	1	1	3	1	7
Eating disorder	0	0	0	1	0	0	1
Total	17	4	11	12	3	3	50

except that they wished that they had more training in using the GMHAT/PC-based interview.

Discussion

The findings were very encouraging as the nurses could easily administer the GMHAT/PC interview in the Arabic population in a reasonable time frame of approximately 16 minutes. The patients easily understood the questions and readily accepted the interview. The feedback from the nurses' interviews was generally very positive, except that they wished that they had more training in using the GMHAT/PC based interview.

Good agreement was found between the psychiatrist's ICD-10 based clinical diagnosis and the GMHAT/PC

interview diagnosis, with good sensitivity and specificity, which makes GMHAT/PC a practical clinical tool for health professionals in Arabic-speaking regions.

Mental health treatment needs of the population remain neglected in the EMR due to inadequate resources but more importantly due to lack of awareness, training and knowledge of health professionals to deal with mental health problems in their communities. Easy access to computers even in remote regions makes it feasible to use computers for routine assessments. GMHAT/PC could therefore fill an important gap in equipping health workers with the skills for diagnosing mental illness in their populations and directing them towards appropriate treatments.

The study had some limitations, particularly in that the number of patients interviewed was small and the interview setting was a hospital setting. Further work is therefore needed in assessing the feasibility and psychometrics of the Arabic version of GMHAT/PC in the primary care and general health setting. However, this small pilot study demonstrates the feasibility and applicability for using GMHAT/PC to diagnose mental illness in this population.

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The GMHAT/PC will be available for free download from: <http://www.gmhath.org>.

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Competing interests: None declared.

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Predictors of smoking among male college students in Saudi Arabia

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المنبئات بالتدخين بين طلاب الجامعات في المملكة العربية السعودية

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الخلاصة: يعتبر التعرف على المنبئات بالتدخين في واحدٍ من البلدان التي تحتل القمة في تدخين السجائر في العالم خطوة بالغة الأهمية في الوقاية من التدخين. وقد أجرى الباحثون دراسة مستعرضة لتقييم منبئات التدخين لدى أترابٍ ذكورٍ من الطلاب في ثلاث جامعات في المملكة العربية السعودية. واستخدم الباحثون استبياناً تم التحقق من صحته واختباره من أجل التعرف على الخصائص الاجتماعية والديموغرافية، والأداء الأكاديمي، وتدخين الزملاء، ووجود مدخنين ضمن الأسرة. وقد شملت الدراسة 337 مشاركاً، وكان 30.9٪ منهم من المدخنين حالياً (دخنوا سيجارة واحدة أو أكثر خلال الأيام الثلاثين المنصرمة)، وكانت المنبئات التي يعتد بها إحصائياً والتي تم التعرف عليها بالنسبة للوضع من حيث التدخين باستخدام تحليل تحوفي لوجستي متعدد المتغيرات هي: الأداء الأكاديمي المنخفض (نسبة الأرجحية 2.29، فترة الثقة 95٪، تتراوح القياسات بين 1.02 و5.17)، وتدخين الزملاء (نسبة الأرجحية 4.14، فترة الثقة 95٪، تتراوح القياسات بين 1.53 – 11.3)، ووجود مدخنين ضمن الأسرة (نسبة الأرجحية 2.77، فترة الثقة 95٪، تتراوح القياسات بين 1.37 و5.64). وتوضح هذه النتائج تأثير ضغط الأسرة والزملاء على البدء بتدخين السجائر بين الشباب السعوديين.

ABSTRACT Identifying the predictors of smoking in one of the top cigarette-consuming countries in the world is a vital step in smoking prevention. A cross-sectional study assessed the predictors of smoking in a cohort of male students in 3 universities in Saudi Arabia. A pre-tested, validated questionnaire was used to determine sociodemographic characteristics, academic performance, peers' smoking, and presence of a smoker within the family. Of the 337 participants, 30.9% were current smokers (smoked 1 or more cigarettes within the last 30 days). Lower academic performance (OR = 2.29, 95% CI: 1.02–5.17), peer smoking (OR = 4.14, 95% CI: 1.53–11.3) and presence of other smokers in the family (OR = 2.77, 95% CI: 1.37–5.64) were the significant predictors of smoking status identified using multiple logistic regression analysis. These findings highlight the influence of family and peer pressure in initiating cigarette use among the youth of Saudi Arabia.

Facteurs prédictifs de tabagisme chez des étudiants de sexe masculin en Arabie saoudite

RÉSUMÉ Identifier les facteurs prédictifs de tabagisme dans l'un des premiers pays consommateurs de cigarettes au monde est une étape essentielle dans la prévention du tabagisme. Une étude transversale a évalué les facteurs prédictifs du tabagisme dans une cohorte d'étudiants de sexe masculin dans trois universités en Arabie saoudite. Un questionnaire validé et prétesté a été utilisé pour recueillir des données sur les caractéristiques sociodémographiques des répondants, leurs résultats universitaires, la présence de fumeurs parmi leurs pairs et dans leur famille. Sur 337 participants, 30,9 % étaient des fumeurs actifs, c'est-à-dire qu'ils avaient fumé au moins une cigarette au cours des 30 derniers jours. Des résultats universitaires plus faibles (O.R. = 2,29 ; IC à 95 % : 1,02–5,17), la présence de fumeurs parmi leurs pairs (O.R. = 4,14 ; IC à 95 % : 1,53–11,3) et dans leur famille (O.R. = 2,77 ; IC à 95 % : 1,37–5,64) étaient les facteurs prédictifs de tabagisme significatifs identifiés à l'analyse de régression logistique multiple. Ces résultats soulignent l'influence exercée par les membres de la famille et la pression placée par les pairs sur les jeunes d'Arabie saoudite pour qu'ils se mettent à fumer.

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Introduction

Smoking is the leading preventable cause of death, responsible for 5.6 million deaths around the world [1–3]. Saudi Arabia is one of the top 10 cigarette-importing countries in the world [3]. The estimated economic, social and health costs associated with all tobacco use in the country was estimated to be \$1.3 billion in 2010, with males contributing the most to this estimate [4]. The prevalence of smoking in Saudi Arabia has been reported to be as high as 52.3%, and among school and university students it has reached an alarming rate of 30% and 50% respectively [5]. Furthermore, comparison of Global Youth Tobacco Surveys of 13–15-year-olds found a 30% increase in smoking prevalence in Saudi Arabian males between 2001 to 2007 [6]. The majority of smokers in Saudi Arabia start smoking before the age of 15 years [5]. The price of cigarettes makes them affordable to most children, and there is no strict application of the minimum legal age for purchasing cigarettes [7]. The reported prevalence among men (13%–38%) is much higher than that among women (1%–16%) [8], presumably due to the local culture and traditions in Saudi Arabia whereby smoking by females is considered shameful [9].

Addressing factors associated with smoking is a crucial strategy for reducing the number of smokers and improving the health of a nation. A considerable amount of literature has been published on the predictors of smoking, but not specifically within the Saudi Arabia population. Variables such as age, income, academic performance, peer pressure and family members smoking have been shown to be significant predictors of smoking around the world [10–15]. In particular, smoking by young adults was a strong predictor of smoking behaviour in adulthood [11]. A study of 1200 young American adults reported that initiation of cigarette smoking at

an early age was associated with higher cigarette consumption, greater nicotine dependence and longer duration of smoking [16].

Cigarette smoking is a major health concern in the male population of Saudi Arabia and the government needs to consider addressing it using behavioural and/or educational interventions. Due to the lack of adequate research in Saudi Arabia on the predictors of smoking behaviour, and considering the cultural differences compared with other nations as well as the high prevalence of smoking in young males, our study sought to identify the predictors of smoking in a cohort of young male Saudi Arabian university students. The goal was to inform strategies that would improve resource allocation to anti-smoking interventions.

Methods

Study design and data source

An observational, cross-sectional study was made to predict smoking among a sample of Saudi Arabian male college students. Data were collected from December 2011 to January 2012 using a pretested, validated, self-administered survey [17]. The survey was distributed in 3 government universities in Saudi Arabia. Two of the selected universities provide general higher education (unspecialized) opportunities. The third institute is a technical college that focuses on computer, engineering and industrial sciences. One of the universities is located in the east of Saudi Arabia (Al Hassa). The second university and the technical college are in the Al Qassim region, which is located in the central part of Saudi Arabia. The total number of students within the 3 institutes was about 70 000.

Convenience sampling strategy with a goal of at least 400 participants was considered in this study. Within the universities, 4 health and 1 computer school agreed to assist in data collection.

The teaching faculty at each institute assisted in distributing the surveys. Surveys were administered to students during the last 15–20 minutes of their lecture. Students were requested by teachers to participate by anonymously filling out a survey about their smoking behaviour and to drop the completed survey in a box that was available in each lecture room. All boxes were collected after the lectures ended. Participation in the study was voluntary, and an informed consent letter was provided before proceeding with the data collection. The survey was approved by the institutional review board at the University of Houston.

Survey design

The questionnaire was developed in English and translated into Arabic language using a translation and back-translation method [18]. The translated survey was validated with the help of 3 bilingual experts and was pretested for reliability using the test–retest reliability method for 10 subjects before commencing the data collection.

The variables considered in this study were part of a larger survey that consisted of 56 questions divided into 7 sections in a 4-page-long survey. In this study, the variables considered were smoking status, age, age first initiated smoking, income, marital status, academic performance, peer smokers and presence of smokers within the family, such as mother, father, brother or any other family member (non-first-degree relative).

Smoking status was the main outcome variable evaluated, and a participant was considered a current smoker if he had smoked 1 cigarette or more within the last 30 days. Age was divided in 4 groups, from 18–20 years to > 26 years. Participants were also asked to report age of initiation of smoking. Income was divided into 2 categories according to maximum financial aid for Saudi students: > 12000 Saudi riyals/year (US\$ 3200/year)

and ≤ 12000 Saudi riyals/year (US\$ 3200/year). The marital status variable was classified as single or married. Students' academic performance was measured by asking the participant what grade they received in school, with 4 choices: A, B, C or D. For peer smokers, respondents were asked if any of their friends were smokers (yes or no). The presence of a smoker within the family was categorized using 4 variables: mother, father, brother or any other smoker at home (non-first-degree relative). Participants were asked if any of their family members were smokers or former smokers (yes or no). These variables were selected based on previous studies and through pilot testing [10–15].

Statistical analysis

Descriptive statistics and bivariate analysis was used to identify the predictors of smoking. Any variable with a probability of 0.2 or less in the bivariate analysis was retained in the final multiple logistic regression. A multiple logistic regression model was performed to determine the predictors of smoking. Data were coded and entered using Microsoft Excel 2010, and data was analysed using SAS, version 9.3.

Results

A total of 467 out of 920 surveys were received from students at the 3 universities, a net response rate of 50.8%. Due to data missing for the main outcome variable, 130 surveys were excluded providing a net response rate of 36.6%. The mean age was 22.2 (SD 2.2) years. The majority (66.5%) of participants were between 21 and 23 years old and single marital status (96.3%) (Table 1). Nearly two-thirds of participants (77.4%) were receiving $\leq$ US\$ 3200/year. Most respondents (79.5%) had scored A or B throughout their academic life. About 78.2% of participants reported that they had at least 1 smoker friend and 33.4%

had at least 1 smoker brother. About 34% of participants indicated that they had 1 or more other smoker members of their family at home (non-first-degree relative).

Almost one-third of participants (104, 30.9%) were current smokers. The average reported age for initiating the smoking habit was 15.0 (SD 4.7) years, with a median of 16 and a range of 8–30 years.

The results of the bivariate analysis of smoking status are summarized in Table 1. Significantly more smokers (29.8%) had a reported income $>$ US\$ 3200/year than did non-smokers (19.3%) ($P = 0.033$). Smokers had significantly lower educational grades than non-smokers, e.g. 29.7% had achieved grade A versus 44.8% of non-smokers ($P = 0.004$). Significantly more smokers than non-smokers reported having at least 1 friend who smoked (92.9% versus 72.3%) ($P < 0.001$), having at least 1 smoker brother (43.7% versus 29.3%) ($P = 0.017$) and having 1 or more smoker non-first-degree relatives at home (48.2% versus 28.4%) ($P = 0.001$).

Table 2 presents the results of the multiple logistic regression of smoking status with all variables that had a significant level of 0.2 or less in the bivariate analysis. The risk of smoking increased about 2-fold (OR = 2.29, 95% CI: 1.02–5.17) with low academic performance (grades C to D). The risk of being a smoker was higher for respondents with friends who smoked (OR = 4.14, 95% CI: 1.53–11.3) and with other smokers in the family (non-first-degree relatives) (OR = 2.77, 95% CI: 1.35–5.64).

Discussion

In our cohort of male university students, the percentage of current smokers, i.e. individuals who smoked at least 1 cigarette during the last month, was 30.9%. This was consistent with 2 other studies that were conducted in

similar geographic areas to our study. One carried out in 2003 on 2203 secondary-school male students in Al Qassim, Saudi Arabia found that 29.8% of respondents were smokers [19]. The second study in 2006 on 1652 secondary male students in Al Hassa, Saudi Arabia found that 30.3% were current smokers [20]. In the current study, the influence of peers and families on smoking behaviour was apparent. Peer smokers such as friends and the presence of other smokers (other than father, mother or brother) at home were significant predictors of smoking status for male college students in Saudi Arabia. Academic performance was associated with smoking status, as low grades increased the risk of smoking.

Smoking in American adolescence and young adulthood has been reported as a predictor of adult smoking [11]. In the current study, age was not associated with smoking status because most smokers in Saudi Arabia begin smoking at an early age (mostly below 15 years) [21–23] and our sample had a narrow focused cohort of only university students. The average age of this sample was 22 years, as the entire sample was recruited from the undergraduate college student population. Thus, the age effect was not, or might not be, captured in this study.

Income in our study was associated with an increase in risk of smoking in the bivariate analysis but was insignificant after controlling for potential confounders in the multiple logistic regression model. This result differs from a survey by Khader et al. in 2005 on 712 university students in Jordan [10]. They found that income increased the risk of smoking among students.

Academic performance was a significant predictor of smoking in our study. Students with lower grades (C to D) were found to have a 2.3 times greater likelihood of being smokers compared with those who had higher (A) grades. The academic performance variable was used in 2 previously reported smoking

Table 1 Demographic characteristics and bivariate analysis of smoking status among Saudi Arabian male college students

Characteristic	Total (<i>n</i> = 337) ^a		Non-smokers (<i>n</i> = 233)		Smokers (<i>n</i> = 104)		<i>P</i> -value
	No.	%	No.	%	No.	%	
<i>Age group (years)</i>							
18–20	50	15.7	39	17.3	11	11.7	0.132
21–23	212	66.4	150	66.7	62	66.0	
24–26	42	13.2	24	10.7	18	19.1	
> 26	15	4.7	12	5.3	3	3.2	
<i>Marital status</i>							
Married	12	3.7	7	3.1	5	5.0	0.391
Unmarried	316	96.3	221	96.9	95	95.0	
<i>Income (US\$/year)</i>							
> 3200	76	22.6	45	19.3	31	29.8	0.033
≤ 3200	261	77.4	188	80.7	73	70.2	
<i>Academic performance</i>							
Grade A	133	40.2	103	44.8	30	29.7	0.004
Grade B	130	39.3	90	39.1	40	39.6	
Grades C to D	68	20.5	37	16.1	31	30.7	
<i>Having a smoker friend</i>							
Yes	233	78.2	154	72.3	79	92.9	< 0.001
No	65	21.8	59	27.7	6	7.1	
<i>Current or former smoker in the family</i>							
<i>All (as a family)</i>							
Yes	116	38.0	90	41.5	26	29.5	0.052
No	189	62.0	127	58.5	62	70.5	
<i>Mother</i>							
Yes	60	19.9	43	20.1	17	19.3	0.890
No	242	80.1	171	79.9	71	80.7	
<i>Father</i>							
Yes	119	39.4	80	37.0	39	45.4	0.182
No	183	60.6	136	63.0	47	54.7	
<i>Brother</i>							
Yes	101	33.4	63	29.3	38	43.7	0.017
No	201	66.6	152	70.7	49	56.3	
<i>Any other smokers in the family</i>							
Yes	99	34.0	59	28.4	40	48.2	0.001
No	192	66.0	149	71.6	43	51.8	

^aThe numbers may not total 337 for some variables due to missing values.

predictor studies [12,14]. Both of these studies found a strong association between low academic performance and smoking, which was consistent with our study findings. The first study was conducted on American students in 1992, and found that lower grades

increased the likelihood of smoking status by 2.6-fold [10]. The second study was done in 2005 on Jordanian students and reported that having grade C was associated with a 4-fold risk of smoking compared with having grade A [12].

Consistent with the reported literature, our study found that having at least 1 smoker friend increased the risk of being a smoker 4-fold. A study in 2006 on middle- and high-school students in Cyprus reported that the strongest predictor of smoking during

Table 2 Multiple logistic regression results of smoking status among Saudi male college students

Variable	OR (95% CI)	P-value
Age group (years)		
18–21	1	
21–23	1.40 (0.59–3.35)	0.448
24–26	1.43 (0.47–4.29)	0.527
> 26	1.11 (0.23–5.29)	0.897
Income(US\$/year)		
> 3200	1.57 (0.81–3.07)	0.186
≤ 3200	1	
Academic performance		
Grade A	1	
Grade B	1.40 (0.71–2.76)	0.328
Grades C to D	2.29 (1.02–5.17)	0.045
Having a smoker friend		
Yes	4.14 (1.53–11.3)	0.005
No	1	
Current or former smoker mother		
Yes	0.56 (0.22–1.39)	0.209
No	1	
Current or former smoker father		
Yes	1.58 (0.80–3.13)	0.185
No	1	
Current or former smoker brother		
Yes	0.91 (0.44–1.87)	0.8
No	1	
Any other current or former smoker in the family		
Yes	2.77 (1.37–5.64)	0.005
No	1	

OR = odds ratio; CI = confidence interval.

both early and late adolescence was peers' smoking status, as it increased the smoking risk by 20-fold [14]. Additionally, a study conducted in 2010 on male Saudi Arabian school students aged 16–18 years reported that having peer smokers increased the risk of smoking by 3.5-fold [24].

A longitudinal study conducted between 1991 and 1994 on 3rd to 8th grade students in the United States reported that having a family member who smoked at home was a significant predictor of smoking at an early age [15]. In our study having 2 smokers in the family, i.e. brother and any other

(non-first-degree relative), was significant in the bivariate analysis. But after adjusting for confounders in the multiple logistic regression model, only 1 variable (other smokers in the family) was significantly associated with the risk of smoking. This suggests that participants in our study were influenced by other members of the family or brothers, namely individuals they could related to in their social context, rather than their father.

A number of limitations need to be considered before applying the findings of this study. Cross-sectional study designs can identify

the association between a predicted variable and the predictors, but cannot establish causality. The generalizability of these findings is limited to similar populations of college students in Saudi Arabia. Finally, this study was done using a convenience sample. Therefore, non-participants were not characterized and the influence of non-participation due to possible reporting of smoking behaviour was not captured.

The results suggest that an educational and consultation programme for families could be effective in reducing the number of smokers. Furthermore, educating students on the harm of smoking and the impact of peers could aid in the prevention of smoking. Starting such educational campaigns at an early age may influence students to not initiate smoking. Educational advertisements that highlight the role of family and the role that friends play in the process can provide added benefit in reducing smoking behaviour in Saudi Arabia.

Conclusions

This study identified predictors of smoking in male college students in Saudi Arabia. We found that lower academic performance, peer smoking and the presence of other smokers in the family were significant predictors of smoking status. Despite application of the WHO Monitor Protect Offer Warn Enforce Raise (MPOWER) framework recommendations in Saudi Arabia, more efforts should be made to protect the youth population in areas where youth reside such as universities.

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Salt intake in Eastern Saudi Arabia

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تناول الملح في شرق المملكة العربية السعودية

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الخلاصة: يرافق تناول مقادير كبيرة من الملح تأثيرات جانبية ضائرة مثل ارتفاع ضغط الدم وأمراض القلب والأوعية. ومن غير المعروف كمية الملح التي يتناولها السكان في المملكة العربية السعودية. وتهدف هذه الدراسة إلى تقدير كمية المتناول من الملح بين سكان المنطقة الشرقية في المملكة العربية السعودية من خلال قياس كمية الصوديوم المفرغ في البول خلال 24 ساعة. وقد جمع الباحثون عينات البول من 130 شخصاً أعمارهم فوق 14 عاماً، وقاسوا مستويات الصوديوم والكهرليات الأخرى. واتضح أن 87 عينة قد وفّت بمعايير الدقة، فتم تحليلها. وكان وسطي إفراغ الصوديوم في 24 ساعة في الفئة المدروسة 140 ميلي مكافئ (بانحراف معياري 49 ميلي مكافئ)، [للذكور 153 (بانحراف معياري 52)، وللإناث 118 (بانحراف معياري 37)]. وتتجاوز هذه القيم المقدار الموصى بتناوله يومياً من الصوديوم، وقد تساهم في خطر الإصابة بارتفاع ضغط الدم والمرضى القلبي الوعائي في المملكة العربية السعودية.

ABSTRACT High salt intake has been associated with adverse side-effects such as hypertension and cardiovascular disease. The amount of salt intake among the population of Saudi Arabia is not known. The objective of this study was to estimate the salt intake among residents of the Eastern region of Saudi Arabia by measuring 24-hour urinary sodium excretion. Urine samples were collected from 130 individuals aged over 14 years for measurement of levels of sodium and other electrolytes. A total of 87 samples met the criteria for accuracy and were analysed. Total mean 24-hour sodium excretion for the group was 140 (SD 49) mEq [153 (SD 52) mEq for males and 118 (SD 37) mEq for females]. These values exceed the recommended daily intake of sodium and may contribute to the risk of developing hypertension and cardiovascular disease in Saudi Arabia.

Apport en sel dans l'est de l'Arabie saoudite

RÉSUMÉ Un apport élevé en sel a été associé à des effets secondaires indésirables tels que l'hypertension et des maladies cardio-vasculaires. La quantité de sel consommée par la population d'Arabie saoudite n'est pas connue. L'objectif de la présente étude était d'estimer l'apport en sel chez des résidents de la région est d'Arabie saoudite en mesurant leur excrétion urinaire de sodium en 24 heures. Des échantillons d'urine ont été recueillis auprès de 130 personnes âgées de plus de 14 ans afin de mesurer les concentrations de sodium et d'autres électrolytes. Au total, 87 échantillons ont satisfait aux critères de précision et ont été analysés. L'excrétion de sodium moyenne totale en 24 heures pour le groupe était de 140 mEq (E.T. 49) (153 mEq [E.T. 52] pour les hommes et 118 mEq [E.T. 37] pour les femmes). Ces valeurs sont supérieures à celles recommandées pour l'apport en sodium quotidien et représentent un risque de survenue d'hypertension et de maladies cardio-vasculaires dans la population saoudienne.

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Introduction

High levels of salt intake in the form of sodium chloride are associated with adverse events such as the development of hypertension, cardiovascular events and strokes [1–4]. Reducing dietary salt intake could substantially reduce cardiovascular events and strokes and may increase people's lifespan and reduce national health-care expenditures [5–9]. The World Health Organization (WHO) has recommended salt reduction as a top priority for tackling noncommunicable diseases and has considered this a public health target [10]. Similarly, the Institute of Medicine (IOM) in the United States has issued recommendations to decrease sodium intake [11].

There is a great variation in salt intake between different populations, ranging from 1 mEq/day among the Yanomamo Indians in the Amazon valley in Brazil to 299 mEq/day in some parts of China [12–15]. The amount of salt intake among the population of Saudi Arabia is not known. The aim of this study was to measure 24-hour urinary sodium excretion in order to estimate the sodium intake and to measure some other essential electrolytes among citizens in the Eastern region of Saudi Arabia.

Methods

Sample

A total of 130 citizens from Eastern Saudi Arabia above the age of 14 years were recruited. The participants included healthy volunteers, healthy potential kidney donors and patients who underwent work-up for nephrolithiasis.

Data collection

Sodium intake was estimated by measuring 24-hour urinary sodium excretion. Samples were collected between October and March for 4 consecutive years between 2009 and 2012. Due

to the hot climate in this area during the summer months and the possibility of excessive sodium loss in sweat, the samples were collected during the season of temperate climate between October and March. The study was approved by the institutional review board at Saudi Aramco Medical Services Organization.

The participants were given clear instructions about urine collection for a total of 24 hours. All participants were instructed not to modify their diet during the study period. Patients with chronic kidney disease, those receiving diuretics and patients with gastrointestinal disorders were excluded. To guard against over- and under-collection, urinary creatinine was measured in all samples. A daily creatinine excretion of 20–25 mg/kg and 15–20 mg/kg lean body weight was expected for males and females respectively. Samples with a total creatinine excretion outside these ranges were rejected. In addition to urinary sodium, other electrolytes including potassium and magnesium were also measured.

Blood pressure (BP), weight, and body mass index (BMI) were determined for all participants. Casual (in office, resting) BP was measured using automatic oscillometric devices following the standardized National Joint Commission protocol [16]. The mean of 3 BP measurements at 3 different encounters was recorded.

The electrolytes were measured by dry chemistry methods using Vitros 350 Chemistry System (Ortho Clinical Diagnostics).

Data analysis

The software *Microsoft Excel* 2010 and *Graphpad Prism*, version 2.0 were used for the statistical analysis. Data were expressed as mean values with standard deviation (SD). Pearson correlation coefficient (r) was used to analyse the association between electrolytes excretion and the various variables of the

study population. P -values of < 0.05 were considered significant.

Results

The 24-hour urinary collection was performed on a total of 130 participants; 43 samples were excluded from the analysis due to incorrect collection. Analysis was performed on a total of 87 samples (54 from males and 33 from females). The mean age of the participants was 44 (SD 18), range 14–83 years. The mean ages were 45 (SD 18) years and 41 (SD 17) years for males and females respectively.

Table 1 shows the demographic data of the participants and the measured electrolyte excretion in male and female participants. The sodium excretion of the male participants was particularly high (153 mEq/day), and much higher than that of the female participants (118 mEq/day).

Correlations between 24-hour sodium excretion and age, BMI and systolic and diastolic BP are shown in Table 2. Among the male participants there was a negative correlation between sodium excretion and age, and a positive correlation with body weight. This did not reach statistical significance, however, for the female participants.

Discussion

The WHO recommends that all countries assess the sodium consumption of the population [17]. Most of the sodium in the diet is ingested in the form of sodium chloride. The WHO recommends an intake of no more than 5 g of sodium chloride or 2 g of sodium (85 mEq) per day, while the IOM recommends that adults should not consume more than 2.3 g of sodium (100 mEq) per day [11,17]. Based on these recommendations and due to the adverse health effects of dietary salt, many countries have adopted policies

Table 1 Demographic data and 24-hour urinary electrolyte excretion of the study participants

Sex	No. of people	Age (years)	BMI (kg/m ²)	Mean (SD) values		Electrolytes		
				Systolic	Diastolic	Na ⁺ (mEq)	K ⁺ (mEq)	Mg ²⁺ (mg)
Total	87	44 (18)	27 (5)	129 (15)	75 (8)	140 (49)	56 (22)	81 (37)
Male	54	45 (18)	28 (5)	132 (15)	75 (8)	153 (52)	60 (24)	88 (41)
Females	33	41 (17)	27 (6)	125 (14)	74 (6)	118 (37)	50 (16)	70 (25)

BMI = body mass index; BP = blood pressure; SD = standard deviation.

Na⁺ = sodium; K⁺ = potassium; Mg²⁺ = magnesium.

to regulate salt consumption through educating people to reduce the use of salt in cooking and by persuading the food industry to minimize the use of salt in their products. Estimating salt intake by measuring salt excretion is an essential step towards formulating these policies. There are different methods for estimating sodium consumption, including 24-hour urinary collection, casual spot urine collection and timed spot urine collection. Among these methods, the first is the most accurate. In an earlier report in 2007, the WHO suggested that as few as 100 individuals from a representative sample, with each participant carrying out a single 24-hour urine collection, would be sufficient to provide an estimate of the sodium intake of a population [18].

The daily intake of sodium has not been officially documented in

the Saudi population. As a result of lifestyle changes in the country, diabetes and hypertension have reached epidemic levels [19]. The hot climate during the summer months in Eastern Saudi Arabia may result in large losses of water and salt in the sweat. To avoid any substantial sodium loss in the sweat, we conducted the study during the season of moderate climate between October and March. The main bulk of the study sample was healthy adult individuals. Sodium intake, as reflected in the mean 24-hour sodium excretion rate, was 140 (SD 49) mEq, which is higher than recommended by both WHO and IOM [10,11]. This was true for both males and females, with mean values of 153 (SD 52) mEq and 118 (SD 37) mEq respectively. There was a negative correlation between sodium excretion and age, and a positive

correlation with BMI among the male participants, most likely as a result of the overall high calorie intake among the young and overweight individuals. This was not observed in the female participants, probably due to the small sample size. We did not find a correlation between sodium excretion and either systolic or diastolic blood pressure. This may be attributed to the relatively young age of the cohort.

Besides sodium intake, low potassium intake has also been associated with the development of cardiovascular disease and stroke [20]. Adequate potassium intake is recommended to counteract the adverse effects of sodium chloride. The IOM recommended a daily potassium intake of 4.7 g (120 mEq) for adults [21]. Similar to sodium, potassium homeostasis is mainly regulated by the kidneys. The amount of potassium loss in the sweat and through the gastrointestinal tract is minimal under normal conditions. Therefore, and similar to sodium, urinary excretion of potassium is considered a surrogate for potassium intake. Our data showing a 24-hour mean excretion of 56 (SD 22) mEq indicated that potassium intake was low in the total group and in both sexes of the sample population [60 (SD 24) mEq and 50 (SD 16) mEq in males and females respectively]. This, in addition to high sodium intake, puts our population at an increased risk for the development of hypertension, cardiovascular disease and stroke.

Table 2 Correlation between 24-hour urinary sodium excretion and several variables among male and female participants

Sex/variable	r	95% CI	P-value
Males			
Age	-0.33	-0.55 to -0.07	0.015
BMI	0.27	-0.01 to -0.50	0.05
Mean SBP	0.01	-0.26 to -0.28	0.94
Mean DBP	0.19	-0.08 to -0.44	0.167
Females			
Age	-0.01	-0.35 to -0.34	0.96
BMI	-0.07	-0.41 to -0.28	0.68
Mean SBP	-0.08	-0.41 to -0.27	0.66
Mean DBP	0.05	0.30 to -0.39	0.78

r = correlation coefficient; CI = confidence interval; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure.

Magnesium is another mineral that has vital biological functions. The recommended dietary allowances of magnesium are 320 mg and 420 mg for adult women and men respectively [22]. Balance studies have shown that the net magnesium absorption is around 50%, with a range from 10%–65% of the total intake depending on the diet [23,24]. In the absence of diarrhoea, most of the absorbed magnesium will be excreted in the kidneys. Based on this, our data of 24-hour magnesium excretion [mean 81 (SD 37) mg] may not necessarily reflect magnesium intake, and we can only speculate that the intake of

magnesium in our cohort is lower than what is recommended by the IOM. However, that can only be confirmed by quantification of the actual content of magnesium in the diet.

This study gives insight into the amount of sodium intake in this region of Saudi Arabia, and suggests that measures should be adopted to improve the dietary habits of the population with the hope to decrease the associated adverse health effects of high salt intake. The results of this study could very well be extrapolated to other neighbouring regions where people share similar cultures and dietary habits.

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Investigating inspection practices of pharmaceutical manufacturing facilities in selected Arab countries: views of inspectors and pharmaceutical industry employees

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دراسة ممارسات التفتيش على مرافق صناعة المستحضرات الصيدلانية في بعض البلدان العربية: آراء المفتشين وموظفي شركات صناعة المستحضرات الصيدلانية

سانجي جارج، رانيا شعبان، شان ساهيل، زهير الدين بابار

الخلاصة: هناك عدد قليل من الدراسات حول ممارسات التفتيش على مرافق صناعة المستحضرات الصيدلانية من وجهة نظر المفتشين ومن وجهة نظر الموظفين في تلك المرافق. وقد أجرى الباحثون في هذه الدراسة المستعرضة الوصفية مسحاً تناول المفتشين والعاملين في ضمان الجودة في أربعة بلدان نامية هي الإمارات العربية المتحدة والمملكة العربية السعودية ومصر والأردن، للسؤال عن ممارساتهم في التفتيش وعن وجهات نظرهم. وقد كان هناك قدر كبير من التفاوت في ممارسات التفتيش بين بلد وآخر وبين العاملين في التفتيش وفي ضمان الجودة ضمن البلد الواحد. وكان التباعد في وجهات النظر يترافق مع آليات الدفع للأجور. وقد كان هناك توافق متبادل بين كل من الفرق وبين المفتشين على قلة توافر الإمدادات لديهم، وعلى أنهم بحاجة إلى تدريب أفضل. وقد بدا للباحثين أن المفتشين لديهم صلاحيات أقل مما هو متوقع من أجل مراقبة صناعة المستحضرات الصيدلانية والأنشطة في الأسواق، ويزيد ذلك سوءاً قلة السياسات التي تدعم اتباع أسلوب أكثر انسجاماً ومنهجية ومعمارية في عملية التفتيش ضمن كل بلد، وفي ما بين بلد وآخر في البلدان النامية.

ABSTRACT There are few studies that explore inspection practices of pharmaceutical facilities from the viewpoint of inspectors and industry employees. In this descriptive, cross-sectional study, inspectors and quality assurance staff from 4 Arab countries – the United Arab Emirates, Saudi Arabia, Egypt and Jordan – were surveyed about their inspection practices and views. There was considerable variation in inspection practices across countries and between the inspectorate and quality assurance staff within countries. Divergence was found in views associated with payment mechanisms. There was mutual agreement by both groups that inspectors were in short supply and that they needed to be better trained. Inspectors appeared to have less authority than expected in order to control pharmaceutical manufacturing and marketing activities. Compounding this was a dearth of policy which would support a more uniform and systematic approach to the inspection process within and across countries.

Enquête sur les pratiques d'inspection des établissements de production pharmaceutique dans des pays arabes sélectionnés : opinions des inspecteurs et des employés de l'industrie pharmaceutique

RÉSUMÉ Les études sur les pratiques d'inspection des établissements pharmaceutiques du point de vue des inspecteurs et des employés de l'industrie sont rares. Dans la présente étude transversale descriptive, des inspecteurs et des membres du personnel de l'assurance qualité de quatre pays arabes, à savoir l'Arabie saoudite, l'Égypte, les Émirats arabes unis et la Jordanie, ont été interrogés sur leurs pratiques en matière d'inspection et sur leurs opinions. Les écarts entre les différentes pratiques d'inspection étaient considérables entre les pays mais aussi entre les équipes d'inspecteurs et de l'assurance qualité dans un même pays. Des divergences ont été constatées dans les opinions sur les mécanismes de paiement. Il a été établi par les deux groupes que les inspecteurs étaient en nombre insuffisant et qu'ils avaient besoin d'une meilleure formation. Les inspecteurs semblaient avoir moins d'autorité que prévu dans le contrôle des activités de production et de marketing de l'industrie pharmaceutique. Ce problème était encore aggravé par l'absence de politiques qui permettraient d'appuyer une approche plus uniforme et systématique du processus d'inspection à l'intérieur des pays et entre les pays.

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Introduction

Despite being profit-making businesses, the first priority of pharmaceutical companies should be to assure the quality of the products they manufacture [1,2]. The process of inspection of pharmaceutical facilities is an activity that is expected to assist with compliance by the industry with internationally recognized guidelines that support good manufacturing practice (GMP). There are many types of audits and inspections (routine or formal, concise or abbreviated, follow-up, special inspections and quality system reviews) and varying roles of individuals within regulatory agencies and the pharmaceutical industry [3,4]. The approaches to inspections can be centred on the process, the product or the system or all of these [3].

Within high-income countries, assessing compliance with GMP is well established and there are set protocols for approaching inspections [5–8]. Inspection has become normal practice and audits are accepted as routine and an important aspect of the supply chain process, as GMP verification is required in order to meet the inspection requirements of export markets. Many pharmaceutical companies in Eastern Europe, the Middle East and Africa are undergoing rapid development in order to meet the requirements of industrialized nations. It is common practice for these companies to consult with independent professionals, either privately or through European Union-funded initiatives, in order to obtain GMP certification. This is required for pharmaceutical companies to submit applications for international authorization, enabling them to progress to export business. There is some dialogue suggesting a lack of trained inspectors in developing countries and consequently that the local pharmaceutical companies face difficulties and delays obtaining GMP certification [4,9]. There have been few studies of this issue, but one conducted in Egypt [9] and a commentary from

China [10] suggested that, when available, inspection procedures in developing countries are less common and more variable relative to inspections in developed countries.

Our study was founded on the need to better understand inspection practices, specifically in the context of developing countries in the Arab world. We are not aware of any previous studies that explored dual-stakeholder views (of inspectorate and industry staff) in a single study. The objective of this study was to describe inspection practices in 4 developing countries from the viewpoint of inspectors and pharmaceutical industry staff.

Methods

Sampling frames

Four developing countries were selected from the Arab Middle East, including 2 high-income countries – United Arab Emirates (UAE) and Saudi Arabia – and 2 low- to middle-income countries – Egypt and Jordan. The rationale for selecting these nations was based on their having an interest in development within Arab nations and having likely access to participants and organizations by the lead researchers and a broad range of current practices which were anecdotally known to occur in these countries.

A purposive sampling strategy was adopted in order to understand the viewpoints of those conducting the inspections and those subjected to the inspection process [11]. Two groups of participants were sampled: inspectors from health regulatory authorities and quality assurance (QA) staff working within pharmaceutical companies.

Survey design

This was a descriptive study carried out from September 2008 to February 2009 and a cross-sectional survey design was implemented in order to collect predominantly quantitative data

[12,13]. Additional qualitative data was collected from inspectors within the 4 countries sampled. It was believed that this survey design would provide optimal data collection and could also help to identify issues for more in-depth future research [14]. Ethical approval was obtained from the University of Auckland human participant ethics committee (reference 2009/003).

Data collection

Survey instruments

Two survey instruments were developed from an initial list of questions that this study aimed to answer, as well as through a synthesis of the relevant literature. Question numbers were reduced via an iterative process involving the research team, with each question critically reviewed. Questions that did not directly contribute to answering the research question were omitted. Effort was made to set out the questionnaire as clearly as possible. This included the use of non-ambiguous instructions, along with a simple, clear and attractive layout [15].

Both survey instruments were developed in English; however, Arabic versions were developed in order to be able to engage more respondents in their native tongue. The survey instruments were predominantly quantitative, although open questions were included in order to be able to explore individual opinion about some aspects of inspection practices [12,15]. The surveys were expected to take between 15 and 30 minutes to complete. The layout involved tick boxes outlining a range of responses for each question.

The survey instrument for inspectors contained 37 questions divided into 9 main sections: demographics, including minimum qualifications and experience; available training and education programmes and satisfaction with these and funding; inspection planning and strategies as well as factors influencing duration of the visit; financial considerations; approaches

to visit, recording observations and inspector authority; guideline use; corrective actions, final inspection reporting, approaches to communication of corrective action and follow-up; communication obstacles that could compromise inspections; and recommendations and suggestions (open question).

The survey instrument for pharmaceutical industry QA staff included 20 questions divided into 5 main parts: preparation planning for an inspection visit; financial budgets for inspections; internal auditing, areas of usual inspection, frequency of self-inspection and format of inspection reports; external auditing, types of communication with regulatory body and final report formats; and an open question asking the respondents for recommendations for increasing the inspection efficiency.

Procedures

Surveys were provided to participants either at the time of the country visit by one of the authors (R.H.) or were posted. Different methods were used to engage participants. A covering letter explained the purpose of the research, and the survey questions were concise and specific. Weekly reminder emails and telephone calls were also made to encourage participants to complete the surveys and to return them as soon as possible [12,15].

Definitions

Although the terms are used interchangeably, for the purposes of this study the term "inspection" [2] was used rather than "audit", because it is the term more commonly used in the countries that participated in this research.

For the purposes of this study, the process of a GMP inspection was deemed to be a systematic and methodical review of a facility [3]. The GMP Institute's standard auditing procedure was adopted as the reference standard for this paper [16].

The 3 main approaches used by inspectors in the developing world are:

(a) forward approaches, i.e. tracing forwards from the raw materials and following the system through the factory to the dispatch warehouse—this is a hypothetical exercise that focuses on physical systems; (b) backward approaches, i.e. tracing backwards from the finished product in the warehouse to review the entire history back through the system—this is a fact-based exercise that focuses on documentation; and (c) random approaches, i.e. starting from points around the factory that appear to be significant and working either backwards or forwards as necessary [3].

Data management and analysis

All data collected from participants who responded in Arabic were translated into English by R.H. and checked as part of the data management QA process. Data entry and analysis was conducted using SPSS, version 17 software. All original English and translated data was entered into a single database (in English format), for ease of analysis. Data entries were double checked by statisticians from the Student Learning Centre at the University of Auckland and from the University of the United Arab Emirates to ensure accurate data entry. SPSS was used to calculate the frequency and percentage of responses to each question, as appropriate. Results were presented as tables and graphs within SPSS. Responses to open-ended questions were analysed and broad themes identified, and the frequency of each theme quantified.

Results

Country of domicile

Completed surveys were received from 32 inspectors: 16 in the UAE, 9 in Egypt, 6 in Saudi Arabia and 1 in Jordan. Survey responses were collected via personal interviews in the UAE and by post or in a few cases by email in the other countries. In addition 23 QA staff within pharmaceutical facilities participated in this study: 10 in Egypt,

8 in Saudi Arabia and 5 in the United Arab Emirates (UAE). Surveys were collected from 15 QA staff by post, from 5 by face-to-face interviews and from 3 by email.

Regulatory context

Inspectors reported that the UAE Ministry of Health [17,18], Jordanian Food and Drug Administration [19] and Egyptian Ministry of Health [9] each have 1 regulatory health authority. Conversely, Saudi Arabia has several (Ministry of Health, Saudi Food and Drug Administration and the Gulf Central Committee for Drug Registrations) [20].

Inspectors' views on roles, process, and delegated authority

Qualification and roles

The majority of inspectors specified that a bachelor of pharmacy degree should be required for the job ($n = 23$, 71.8%) and 9 participants (28.1%) suggested that inspectors should be required to pass a national examination of pharmacy or an equivalent certificate. Three respondents (9.3%) noted that there were no specific requirements to become an inspector in their countries. Along similar lines 4 respondents (12.5%) indicated that no specific qualification was required for inspectors; the caveat being the need to have 2–5 years of experience of conducting inspections and the appropriate level of training.

Inspectors' own work experience

Most of the respondents from Egypt, UAE and Saudi Arabia had up to 5 years of experience ($n = 19$, 59.4%), while the single respondent from Jordan reported more than 15 years of experience. The large majority of respondents saw their main role as policing ($n = 28$, 87.5%), as well as the review of required corrective actions ($n = 25$, 78.1%). Nearly two-thirds ($n = 21$, 65.6%) suggest that providing advice and/or consultation and cooperation with industry staff was a key part of their role. A similar

response pattern was seen from representatives in Egypt, although the role of the inspector working with industry staff was given more emphasis than in the UAE. The most common response from respondents from Saudi Arabia was the policing role, followed by working with the industry to improve quality. The inspector from Jordan indicated that all 4 roles were important.

Inspection plans and procedures

Nearly half of the inspectors ($n = 29$, 48.2%) reported a warning period of up to 1 month prior to inspection, while almost one-third ($n = 9$, 31.0%) reported that no announcement was made at all. Extended warnings of up to 12 months occurred less commonly ($n = 3$, 10.3%). Inspectors from Saudi Arabia showed the widest range of time frames from up to 1 month (30.0%), 3 months (33.3%) and 6 months (16.6%). In terms of influencers of the duration of inspection visits, most inspectors reported that the purpose of the visit ($n = 26$, 81.2%) and the size of the company being inspected ($n = 16$, 81.2%) were the main influencers. Other factors important to the inspectors included distance of pharmaceutical facility from main centres and the extent to which "negative" issues were observed during the visit.

Close to two-thirds of inspectors ($n = 20$, 64.5%) reported that an information pack was supplied by the company prior to inspection. Inspectors in the UAE were more likely to have additional information provided by health

authorities ($n = 10$, 62.5%). Access to inspection records from previous visits was less common, particularly in Saudi Arabia where none of the respondents suggest this occurs.

With respect to the inspection visit, the backward and forward approaches were used by similar proportions of inspectors (46.9% and 50.0% respectively), while the random approach was less commonly used (25.0%). Respondents could provide more than one answer to this question and the UAE inspectors reported use of all approaches in equal measure. Saudi Arabia inspectors reported that the forward approach was the only one used. The majority of inspectors from Egypt preferred to take a backward approach to the inspection process. The respondent from Jordan reported that the backward approach was the only one taken (Table 1).

Nearly two-thirds of the inspectors ($n = 19$, 59.3%) indicated that the purpose of the inspection visit was the most important factor when deciding which approach to adopt. The inspection history was also deemed important ($n = 12$, 37.5%), followed by inspector's personal choice ($n = 8$, 25.0%), while some suggested all of the above reasons influenced the decision ($n = 5$, 15.6%). During the inspection, checklists were most commonly used ($n = 26$, 81.2%), but note-taking ($n = 9$, 28.1%) and still cameras were also used by some inspectors ($n = 9$, 28.1%) and a few used flow-charts ($n = 2$, 6.3%) and video ($n = 4$, 12.5%). There was some variation

across countries, with inspectors from UAE using the whole range of available methods.

Corrective actions and final reporting

The majority of inspectors reported that they supplied a description of the inspection list ($n = 21$, 65.6%) and included negative and positive observations in their reports ($n = 20$, 62.5%). Approximately half ($n = 15$, 46.8%) of the respondents included recommendations for improvements ($n = 16$, 50.0%), required corrective actions and the time-frame for required response ($n = 15$, 46.8%) as part of their normal practice.

It would appear that inspectors from UAE and Egypt provided the broadest range of information in their final reports; Saudi Arabia and Jordan less so. Three-quarters of respondents use formal written letters as inspection follow-up on required corrective actions ($n = 24$, 75%). Email was used least often ($n = 3$, 9.3%); telephone and fax were used to the same degree ($n = 12$, 37.0%). Similar patterns of communication methods were reported across the countries studied. The great majority of inspectors reported using a combination of written report and follow-up visit to check that corrective actions had been implemented ($n = 23$, 71.8%). Close to half the inspectors reported using a follow-up visit ($n = 15$, 46.8%) and more than one-quarter ($n = 9$, 28.1%) requested company reports; outlining that corrective actions had occurred.

Table 1 Approaches to inspection of pharmaceutical manufacturing facilities used by inspectors in the 4 countries

Country (no. of inspectors responding)	Forward approach ^a		Backward approach ^b		Random approach ^c	
	No.	%	No.	%	No.	%
United Arab Emirates ($n = 16$)	8	50.0	8	50.0	8	50.0
Saudi Arabia ($n = 5$)	5	100.0	0	0.0	0	0.0
Egypt ($n = 9$)	2	22.2	7	77.7	0	0.0
Jordan ($n = 1$)	0	0.0	1	100.0	0	0.0
Total ($n = 32$)	15	46.9	16	50.0	8	25.0

Respondents could provide more than 1 answer to this question.

^aTracing forward from raw materials and through the factory to end at the dispatch warehouse; ^bTracing backward from finished product in the warehouse to review history back through the system; ^cStarting from points around the factory that appear to be significant and working either backward or forward as necessary [6].

Table 2 Inspectors' views on inspection issues and difficulties

Issue or difficulty ^a	No.	% (<i>n</i> = 32)
Lack of training and education programmes	13	40.6
Inadequate numbers of inspectors	9	28.1
Transportation difficulties	9	28.1
Not enough time to complete the required inspection	7	21.9
Difficulties in arranging the time of inspection between inspectors and facility	5	15.6
Inadequate salary and benefits for inspectors from the regulatory authorities	5	15.6
No specific format for reporting observations and findings	5	15.6

^aItems mentioned by > 15% of respondents.

Inspector authority

Just over half (*n* = 17, 53.1%) of the inspectors surveyed reported that they had the authority to delay the issue of a GMP certificate and 3 respondents (9.3%)—1 from each of UAE, Saudi Arabia and Jordan—reported the ability to revoke marketing authorization. Less than one-quarter (*n* = 7, 21.8%) of inspectors were in a position to close a facility or delay approval of licenses or marketing authorizations.

Guidelines from the Therapeutic Goods Administration in Australia were most commonly referred to (*n* = 18, 56.2%), followed by the European Medicines Agency (*n* = 13, 40.6%) World Health Organization (*n* = 10, 31.2%), United States Food and Drug Administration (*n* = 7, 21.8%) and International Conference on Harmonization (*n* = 3, 9.3%).

Inspection barriers and difficulties

Only 12 of the 32 inspectors (37.5%) responded to the question about communication with overseas inspectors. Of these half (*n* = 6, 50.0%) reported that they had poor communication with international inspectors, the highest being in the UAE. Reported barriers from inspectors included: language (*n* = 15, 46.8%), cultural differences (*n* = 10, 31.2%), inappropriate body language (*n* = 5, 15.6%), lack of communication between inspectors and pharmaceutical industry staff (*n* = 16, 50.0%). Inspectors identified with a long list of issues and difficulties associated with inspections and the more common ones (> 15%) are outlined in Table 2. The most common were lack of training and education programmes, followed by transportation difficulties

to the facilities, inadequate numbers of inspectors and insufficient time set aside to complete the requirements for a thorough inspection.

Levels of response by QA staff to the question about inspection difficulties was very low; there being a single response for most categories. No warning of inspection, no specific inspection plan, lack of allocated time, inspectors without appropriate background and imprecise questioning were highlighted as difficulties.

QA staff views on preparation, process and recommendations

Inspection visit preparation

The majority of QA staff had experienced an inspection visit (*n* = 21, 91.3%). A large majority of respondents agreed that maintenance records (*n* = 18, 78.6%), sanitation and hygiene records (*n* = 15, 65.2%) and standard operating procedures (*n* = 14, 60.9%) were the types of documents to be prepared prior to an inspection visit. A minority of respondents suggested that recall records (*n* = 9, 39.1%) and manufacturing batch records (*n* = 2, 8.7%) should be prepared.

Levels of self inspection

All QA staff noted that facilities had self-inspection plans and a great majority (*n* = 20, 87.0%) had completed written self-inspection plans. Quality control procedures were seen as critical in the self-inspection procedure and there was a long list of them (Table 3).

Table 3 Quality assurance staff responses to question about levels of self-inspection for good manufacturing practice (GMP)

Aspects of GMP for self-inspection	No.	% (<i>n</i> = 23)
Personnel working in the facility	19	82.6
Maintenance of the factory	14	60.9
Manufacturing and testing	17	73.9
Quality control procedure	22	95.7
Documentation preparation	19	82.6
Recall procedures	15	65.2
Follow-up to previous self-inspections	14	60.9
Validation and monitoring procedures	15	65.2
Control of printed components	16	69.6

Table 4 Quality assurance staff responses to question about the components of a final inspection report

Components	No.	% (<i>n</i> = 23)
Brief summary	8	34.8
Report with commentary	6	26.1
Detailed report	11	47.8
Recommendations for improvements	22	95.7
Corrective plans for overcoming non conformity	21	91.3
A time-frame for required responses	20	87.0
Description of the inspection list	21	91.3
Negative and positive observations	20	87.0
Inspectee signature space when corrective action completed	0	0.0

Monthly self-inspection was the most common system, followed by quarterly then 6-monthly. Respondents suggested that corrective actions from a self-inspection were completed within a specified time-frame ($n = 21$, 91.3%); most commonly within 2 weeks to 1 month ($n = 8$, 34.7%), although there was a broad range of responses.

External inspections and recommendations

QA staff views about the use of video or photographs was split relatively evenly between agreement ($n = 11$, 47.8%) and refusal ($n = 12$, 52.2%). Written formal letters were most commonly used to communicate with inspectors ($n = 13$, 56.5%), followed by telephone calls ($n = 8$, 34.8%), emails ($n = 8$, 34.8%) and fax

($n = 6$, 26.1%). Participants' responses to a list of components included in a final inspection report are outlined in Table 4.

Other items concerning observations, recommendations, description of the inspection list and time-frames for the required response all had response rates > 85%. Just under half of the respondents ($n = 10$, 43.4%) suggested that sharing of the final inspection report was conditional and could depend on the results in the report and the reason why another authority had asked to view it. A minority of participants would share their inspection reports with other authorities ($n = 4$, 17.5%) and the rest would not ($n = 9$, 39.1%).

Education and training

Most of the inspectors reported that training sessions were available for them ($n = 23$, 71.9%). Half suggested an internship for new inspectors was available in some cases but that there were no practical aspects included with the theoretical sessions. Training programmes are compulsory in the UAE and Jordan. The majority of inspectors from the UAE and Jordan were satisfied or extremely satisfied with the QA training programmes. In contrast, the majority of respondents from the Saudi Arabia and Egypt were not satisfied or unsatisfied with the programmes in general (Figure 1).

Inspectors without appropriate background and imprecise questioning were highlighted as difficulties associated with inspections from the viewpoint of QA staff.

Inspection fees

From the viewpoint of inspectors, fees were most commonly of the fixed-fee type. From the viewpoint of QA staff, variable inspection fees were deemed to be most common (Table 5). The inspection fee was most commonly paid by the pharmaceutical company (Table 6).

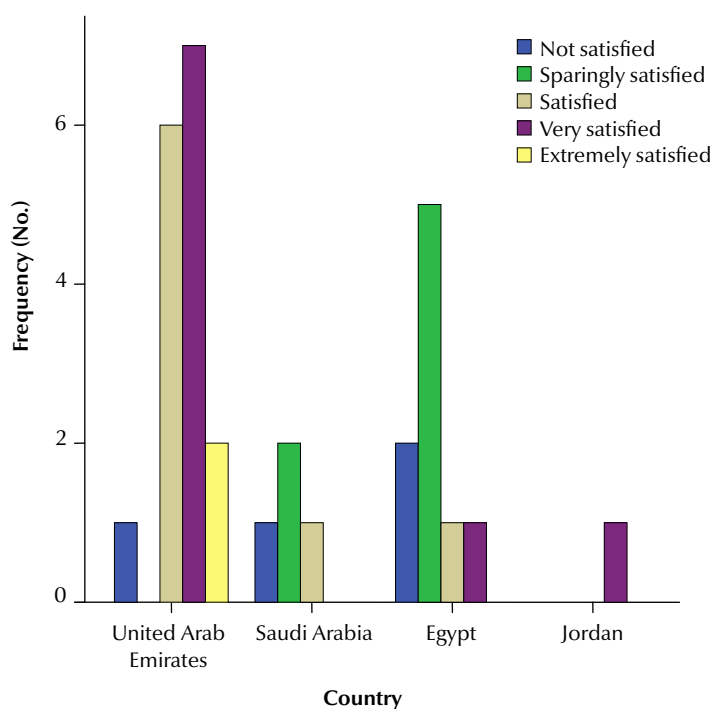
**Figure 1 Satisfaction of inspectors in the 4 countries with training sessions**

Table 5 Inspectors' and quality assurance (QA) staff responses to question about inspection fees

Type of fee	Inspectors (n = 32)		QA staff (n = 23)	
	No.	%	No.	%
Contract (fixed fee)	10	31.3	8	34.8
Confidential or unknown	9	28.1	2	8.7
Variable fee	2	6.3	13	56.5
No specific fee	-	-	2	8.6
According to the regulatory body	-	-	1	4.3

Respondents could provide more than 1 answer to this question.

Only 3 QA staff from different countries reported that fees were set by government. One inspector from the UAE advised that the health authority paid the inspection fee and 1 inspector from Egypt responded that the cost was shared between the pharmaceutical company and the government regulator. Responses to the inspection fee question for QA staff were evenly split between being paid for by the pharmaceutical company or jointly by the company and the regulating health authority.

Discussion

This study set out to investigate the views of both inspectors and QA staff in the context of inspection of pharmaceutical manufacturing facilities in 4 Arab countries.

Inspectors' views

It was expected that inspectors be pharmacists or have considerable experience.

The main role was deemed to be "policing". The level of practical training, approaches and satisfaction with these programmes varied markedly across the 4 Arab countries surveyed. Inspectors believed they had a reasonable level of authority/power and were able to delay GMP certification if they saw fit. The ability to cease manufacturing and product marketing outright was less common.

A range of guidelines were used during the inspection but the Australian Therapeutic Goods Administration guidelines seemed to be most popular. Either fixed fees or fees that were confidential appeared to be commonplace. Inspection follow-up was generally in the form of letters and only half of the respondents made recommendations for improvement. There would appear to be significant variability in the information provided in inspectors' final reports; however, post-inspection follow-up visits appeared to be part of routine practice. Difficulties with inspection

mentioned included lack of time, funding, transportation and standardization of reporting.

QA staff views

There appeared to be consistency across countries in the perceived requirements for the preparation of inspection visits. Furthermore, self-inspection plans and practices were commonly reported and corrective actions were followed within a time-frame. Variable inspection fees were reported to be the most common type, followed by fixed fees. Letters were the most common form of communication with inspection agencies. QA staff expected to receive recommendations for improvement, corrective plans, a time-frame for responding, description of the inspection list and both positive and negative observations. Sharing of reports appeared to be conditional on the findings of the inspection. Difficulties with inspections from the viewpoint of QA staff included: *ad hoc* visits, lack of specific pre-inspection plans, inadequate time spent conducting the inspection and inspectors lacking background about departments within the industry.

Contribution made by this article

Academic literature addressing pharmaceutical inspection processes within the developing world are scarce, despite an increasing trend toward the globalization of pharmaceutical regulation [21,22]. There is commentary

Table 6 Inspectors' and quality assurance (QA) staff responses to question about source of inspection fee payments

Source of staff inspection fee payments	Inspectors (n = 32)		QA staff (n = 23)	
	No.	%	No.	%
Pharmaceutical company	14	43.8	9	39.1
Regulating health authority	1	3.1	3	13.0
Joint payment by pharmaceutical company and regulators	1	3.1	9	39.1
No answer or don't know	6	18.8	2	8.7

Respondents could provide more than 1 answer to this question.

suggesting a lack of trained personnel and significant barriers to ensuring inspection processes are consistent within and across several developing countries. The findings from this study reflect that rhetoric. We are also unaware of studies which compared the views of inspectors and representatives of pharmaceutical companies within the same study cohort; this manuscript adds to that understanding. There appears to be a level of disconnect between inspectors and QA staff with regards some aspects of inspection; both within and across the Arab countries studied. By taking this approach across countries and through key stakeholder viewpoints, misalignment of certain aspects of policy and/or practice have been uncovered which warrant further exploration. For example, in this study divergence was found in viewpoints associated with payment mechanisms. Equally, inspectors needed to be better trained.

Limitations of the research

As with any research this paper has limitations and the results need to be interpreted in the light of these. The sample size was small for both surveys and the analysis was limited to basic descriptive statistics. Due to the small sample size, generalizability of the data may to other contexts not be appropriate because the respondents may not be representative of the populations of QA staff and inspectors. However, the data set was still useful as a descriptive analysis, uncovering issues for further in-depth study [14]. Another limitation was that some participants did not answer all of the questions and declined to provide a reason for their non-response. However the reason may have been concerns about providing confidential information, rather than lack of knowledge.

To increase generalizability across all Arab countries and allow cross-country comparisons, future research

could include more participants from a larger range of countries. This may require a longer period for data collection and could be more expensive to conduct because of telephone calls and the requirement for personal visits to increase engagement. In addition, the involvement of participants from more countries may require translation into multiple other languages.

Implications and recommendations

The aim of this research was to explore inspection practices within the context of 4 Arab countries in order to understand where changes need to be made to increase consistency, efficiency and effectiveness [23]. It is important to consider the implications of the findings for policy, practice and any future research that may be required. Equally important is the need to outline recommendations which can be adopted by authorities and/or the pharmaceutical industry within developing countries.

Implications for policy and practice

The findings of this study have implications for policy and practice when considered from the viewpoint of both inspectors and QA staff (Tables 7 and 8). There is some policy which supports more systematic approaches to inspection of pharmaceutical facilities within the context of developing countries; however, our study showed there were major barriers to translating this into transparent and consistent practice. What was striking was the variability both across and within countries involved in this study. Policy development and implementation should span collaborative approaches to education and training, delegated authority, payment mechanisms and remuneration and policies that support and stimulate the use of technology. In

addition, more operational level policies are required to inform and support technical aspects of the role including standard operating procedures and reporting templates.

In terms of practice, there are expected “flow-on” effects from improvements in policy development and implementation. For example, in the longer term, inspection practices are expected to be better supported through educational policies that attract pharmacy students and through providing basic training with a view to their continuing a career in this area. Local and international peer review and benchmarking practices will assist in standardizing practice and reducing the variability in approaches to the inspection, reporting and follow-up. There were several inconsistencies between the views of inspectors and QA staff on consistent payment practices and the expectations of the inspection process and reporting. Further work is required to better understand and to minimize these differences through practices informed by policy.

Implications for future research

A future research agenda has been developed by identifying gaps in the academic literature alongside the findings of this study (Tables 7 and 8). The following research streams represent the broad concepts in this area of study which require further work.

Stream 1: The influence of further policy development and implementation

Continued development of effective policy needs to occur and its influence evaluated. This would include educational policy promoting the role of inspectors and QA staff, evaluation of training programmes for inspectors, and interventions to reduce variability of the inspection process in its entirety. The impact of international knowledge sharing and collaborations needs to be assessed.

Table 7 Implications of the study for policy, practice and research: perspectives of inspectors

Findings	Implications for policy	Implications for practice	Implications for future research
Inspectors should be pharmacists or have considerable experience. There is a shortage.	Policy to be developed to reflect this view. Joint policy to be developed between health authorities and pharmacy schools	Review of pharmacy degrees in developed countries to ensure the degree provides adequate basic training. Focus on attracting pharmacists to this role	Need for demographic studies to determine the inspection workforce. Understanding the barriers and facilitators to being an inspector.
Perceived as a policing role	Policy to outline other important roles in addition to policing	Change in practice to ensure that the role is not undertaken exclusively as a policing role	The influence of a dominant focus on policing to be further explored in terms of the overall process
Variable levels of practical training in addition to theoretical aspects	Educational policy to be developed which outlines what constitutes effective theoretical and practical training	Practice better supported through practical on job training in addition to the theoretical understanding required	Evaluation of training interventions
Approaches to inspection and process vary markedly along with information provided	Policy to be developed which informs standard inspection	Sharing of reports among inspectors to encourage learning and reduction in variability in amount and format of information delivered	Implementation and evaluation of approaches to reduce inspection variability
Inspectors believe they have adequate power/ authority	Policy required around delegated authority and levels at which inspectors can act. Inability of inspectors to stop marketing authorization and close facilities in some countries negates the point of the inspection	Local and international peer review of decisions regarding inadequate facilities to make the process more robust	Audits of decision and decision-making processes to reduce variability Understand the power differential between inspectors and QA staff and higher management of pharmaceutical facilities and appropriate decision-making
Inspection fees are mostly confidential or fixed: different from QA staff views	Transparent payment policies are required	Consistent and transparent payment practices required	Wide-scale surveys to better understand current payment mechanisms within and across developed countries
Communication: • by letter • language barrier when communicating abroad	Inspection policies need to support the increased use of technology for communicating, producing checklists and reports. Development of cross-country policies for countries of a similar nature. Consistent policy around the use of video and photographs is required.	Internationally recognized standards need to become common practice through interaction between inspectors and authorities in developed and developing countries	Implementation and evaluation of cross-country information-sharing and experiential initiatives
Difficulties with inspections • lack of training or variable training across countries • transportation • insufficient time • salary/reimbursement • no set format for inspection or report	Development of SOPs and written report template formats needed. Training and development policy required to inform compulsory inspector training consistent across developing countries. Work towards international accreditation. Formal examination and licensing policies consistently required across all developing countries	Comprehensive training and educational programmes implemented for inspectors. Periodic inspectors meetings to organize work, reduce deficiencies and outline responsibilities. Links needed between regulatory authority administrators and inspectors in terms of the process, reports and forms used and decision-making processes. Meetings between inspection team members before starting the visit to ensure the inspection team is well coordinated with regard to purpose and individual responsibilities.	Evaluation of the impact of training policy implementation and practices in a before and after study

QA = quality assurance; SOP = standard operating procedures.

Table 8 Implications of the study for policy, practice and research: perspectives of quality assurance staff

Finding	Implications for policy	Implications for practice	Implications for future research
Visit preparation and self-inspection activities undertaken	There is the belief that visit preparation and self-inspection activities are being undertaken, but is no policy around what these procedures should constitute	Although staff believe this aspect is done relatively well, there is always room for improvement, and support of best-practice needed. More information may need to be provided by pharmaceutical facility visits undertaken by international inspectors	Wider evaluation and audit of whether visit preparation and self inspection practices are in fact as common as this study suggests. Understanding what is undertaken and what contribution self-inspection makes so as to inform future best practice in the developing world
Inspection fees are variable, followed by fixed-fees, and this is different from the inspectors	Payment policies required	Consistent and transparent payment practices required	Further exploration of the different views of inspectors and QA industry staff with respect to payment is warranted
Inspection communication and recommendations	Policy to facilitate the use of IT required. QA staff have ideas about what they expect to receive and this needs to be considered for future policy development. Policy around inspection report sharing is required	The gap between what QA staff expect to receive, what is policy and normal practice to be aligned	Wide-scale evaluation of external inspection reports through content analysis would help to inform policy and improve current practice
Difficulties with inspections • <i>ad hoc</i> visiting • planning • time • inspector insight/experience	Policy on <i>ad hoc</i> visiting required. Pros and cons need to be considered. Visits by inspectors from authorities outside of the country less likely to be <i>ad hoc</i> ; joint policy may need to be developed in light of this. Training and development policy required to inform inspector training	More information provided by the pharmaceutical facility prior to inspection, particularly with foreign inspectors. Health authorities websites needed for the industry staff to understand GMP requirements. This practice to be put in place and supported by regulatory policy. Workshops to be provided by regulatory authorities for pharmaceutical facilities to explain GMP compliance and marketing authorization requirements. Appropriate amounts of time needs to be allocated by inspectors and QA staff	Wide-scale surveys of the barriers and facilitators to efficient and effective inspection practices needed based on the findings of this study

QA = quality assurance; IT = information technology; GMP = good manufacturing practice.

Stream 2: Understanding human factors

A better understanding of the current workforce and future potential workforce is required. Alongside this, further work on remuneration packages for inspectors is required through local and international benchmarking. The reasons for the dominant policing culture and how other aspects of the process might be better integrated warrants further exploration. Understanding aspects of decision making and how

inspectors rate sites and feedback to them will allow benchmarking to take place.

Stream 3: System development, operational research and audit evaluation

Undertaking a content analysis of current inspection reports would provide a baseline for measurement of future policy implementation/initiatives. Following on from this, wide-scale studies of the barriers and facilitators of change in this sector are required.

Stream 4: Better understanding of fiscal mechanisms and their influence

There seems to be some misalignment with the experiences of QA staff and inspectors when it comes to fee payment mechanisms. Local and international benchmarking will be required.

Conclusions

This study set out to investigate inspection practices of pharmaceutical

manufacturing facilities in 4 Arab countries through the viewpoints of inspectors and QA staff. The findings of this study have significant implications for policy and practice. There seems to be considerable variation

in the policies and practice of inspection. A future research agenda is posed around 4 streams of work involving human factors, systems and processes alongside fiscal considerations.

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Pharmacovigilance in Qatar: a survey of pharmacists

K. Wilbur¹

التيقُّظ الصيدلاني في قطر: استبيان للصيادلة

كيرى ويلبر

الخلاصة: تمس الحاجة لبرامج وطنية فعالة للتيقُّظ الصيدلاني من أجل رصد التأثيرات الضائرة للأدوية على السكان المحليين. وقد هدفت هذه الدراسة إلى وصف المعارف والخبرات والمواقف والعقبات المدركة بالنسبة للإبلاغ عن التأثيرات الضائرة للأدوية من قبل الصيادلة في قطر. وقد أجاب 116 صيدلانياً على استبيان يتضمن 27 بنداً عبر الإنترنت (معدل الاستجابة 25٪)، وقد كانت المعرفة بالمصطلحات وبالغرض من الإبلاغ بالتأثيرات الضائرة للأدوية مرتفعين، إلا أن 29.3٪ فقط منهم لم يكتب أبداً بلاغاً حول التأثيرات الضائرة للأدوية في قطر. وقد عبّر معظم المستجيبين عن مواقف إيجابية نحو دور الصيدلي في التيقُّظ الدوائي، كما نظر المستجيبون إلى عدم القدرة على التعرف على احتمال وقوع التأثيرات الضائرة للأدوية أو الوصول إلى نموذج الإبلاغ على أنها من العوائق، كما نظر المستجيبون إلى التدريب المعزول وإلى الكفاءة في تقديم البلاغات بمثابة عوامل تسهيل الإبلاغ في المستقبل. وكان الصيادلة في المستشفيات أكثر احتمالاً للإبلاغ عن التأثيرات الضائرة للأدوية بسبعة أضعاف غيرهم في قطر. ويرغب الصيادلة في قطر في الإسهام بأنشطة التيقُّظ الصيدلاني إذا تمّ دعمهم بمزيد من التدريب وبالشفافية في عملية الإبلاغ.

ABSTRACT Active national pharmacovigilance programmes are needed to monitor adverse drug reaction (ADR) data in local populations. The objective of this study was to describe the knowledge, experiences, attitudes and perceived barriers to reporting of suspected ADRs by pharmacists in Qatar. A 27-item web-based survey was answered by 116 pharmacists (25% response rate). Knowledge of ADR terminology and reporting purpose was high, but only 29.3% had ever made a suspected ADR report in Qatar. Most respondents expressed positive attitudes towards the pharmacist's role in pharmacovigilance. Inability to recognize a potential ADR or access a reporting form were perceived as barriers. Enhanced training and efficiency in report submissions were identified as facilitators to future participation. Hospital pharmacists were 7 times more likely to have reported a suspected ADR in Qatar. Pharmacists in Qatar are willing to engage in pharmacovigilance activities if supported by increased training and transparency in the reporting process.

Pharmacovigilance au Qatar : enquête auprès des pharmaciens

RÉSUMÉ Des programmes de pharmacovigilance nationaux actifs sont requis pour surveiller les données relatives aux réactions indésirables aux médicaments dans les populations locales. L'objectif de la présente étude était de décrire les connaissances, les expériences, les attitudes et les obstacles perçus en matière de notification des réactions indésirables par les pharmaciens au Qatar. 116 pharmaciens ont répondu à une enquête en ligne à 27 items (taux de réponse de 25 %). Leur niveau de connaissances en ce qui concerne la terminologie pour les réactions indésirables et les objectifs de notification était élevé, mais seuls 29,3 % d'entre eux avaient déjà notifié une suspicion de réaction indésirable au Qatar. La majorité des répondants ont présenté des attitudes positives au sujet du rôle du pharmacien en matière de pharmacovigilance. L'incapacité à reconnaître une réaction indésirable potentielle ou à accéder à un formulaire de notification ont été perçus comme des obstacles. Une formation et une efficacité accrues dans la transmission des notifications ont été identifiées comme des facteurs favorisant une future participation. Les pharmaciens hospitaliers étaient sept fois plus susceptibles d'avoir notifié une suspicion de réaction indésirable que les autres pharmaciens dans le pays. Les pharmaciens au Qatar sont disposés à s'impliquer dans des activités de pharmacovigilance s'ils bénéficient d'une formation et d'une transparence accrues pour le processus de notification.

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Introduction

Suspected adverse drug reaction (ADR) reporting is the cornerstone of pharmacovigilance activity; however, its infrastructure varies throughout the world. Surveillance programmes within individual health care facilities may supplement a central national registry, which may in turn augment an international database. Most reporting systems are voluntary and while spontaneous reporting offers advantages of low expense and less complexity, barriers such as time, ambiguity in ADR identification and lack of feedback contribute to under-reporting in several countries [1–6].

Qatar is an affluent Arab emirate with a population of 1.9 million (predominantly expatriates). The Qatar Supreme Council of Health (SCH) has a pharmacy and drug control department subdivision assuming various medication regulation roles, but there is no coordinated national pharmacovigilance programme. A recent inventory of pharmacovigilance activity in Qatar inpatient settings found that suspected ADR reporting policies and procedures are in place within all public hospitals, but in only 1 of the 5 private hospitals [7].

The success of any surveillance system relies on the active participation of its reporters and is the responsibility of everyone involved in the medication use process. Pharmacists working in Qatar are a multinational group, emerging from heterogeneous curricula and training programmes abroad, who may have been exposed to different processes of suspected ADR reporting and experiences with pharmacovigilance activities in general [8]. The objective of the present study was to describe pharmacists' knowledge, experiences, attitudes and perceived barriers to ADR reporting in Qatar.

Methods

Sample

Using workplace contact information, all known pharmacists in Qatar ($n = 568$) were invited by email to participate in an anonymous web-based survey. The research was approved by both the University of Qatar and London School of Hygiene and Tropical Medicine institutional review boards.

Questionnaire development

A comprehensive review of the English language literature was conducted using pertinent electronic health databases (*PubMed*, *Embase*, *International Pharmaceutical Abstracts*, *Cumulative Index to Nursing and Allied Health Literature*) from 1990 to December 2010 using a combination of predetermined keywords and phrases. Hand-searching of references of retrieved articles was also performed. The questionnaire was developed according to the domains of interest evaluated in this existing literature: subject demographics; ability to detect suspected ADRs (knowledge); experiences reporting suspected ADRs; attitudes towards the pharmacists' role in ADR reporting; perceived barriers and facilitators to suspected ADR reporting; and recommendations for improvements in this process locally. The questionnaire draft was formatted as an electronic survey and reviewed for face and content validity and piloted by a small randomly selected group of Qatari pharmacists.

Analysis

Incomplete surveys were analysed if a response to the dependent variable question (history of suspected ADR reporting in Qatar) was given. Frequencies of correct answers to ADR knowledge questions were assessed. Responses were further stratified according to categorical demographic parameters as well as comparisons between ADR reporters and non-reporters. Univariate

and multiple logistic regression analyses were used to examine differences in ADR reporting (dependent variable) among pharmacists according to *a priori* defined criteria including independent variables: age; sex; years in practice; and practice setting. All data analyses were conducted using *SPSS for Mac*®, version 19.0.

Results

Background characteristics

The survey remained open between 30 April and 30 June 2011. Of the 142/568 responses (25.0% response rate), 116 (81.7%) surveys included information about prior experiences with reporting suspected ADRs.

A total of 17 different countries of origin were represented and almost half of pharmacists had practised in Qatar for < 5 years (Table 1). Most respondents represented hospital inpatient practices (64.0%). Only 14 (12.1%) had never worked in a hospital pharmacy.

Knowledge of ADRs

Pharmacists' knowledge of ADR terminology was assessed and over 90% identified the World Health Organization description of an ADR; however, approximately 1 in 5 selected statements were inconsistent with accepted ADR descriptions. Most pharmacists were able to correctly distinguish an ADR from a medication error [9,10].

Experience of ADR reporting

Less than half of the respondents (49, 42.2%) had made suspected ADR reports in the past and 34 (29.3%) reported doing so in Qatar. Most of these local reports (29, 85.3%) were by hospital pharmacists, 4 (11.7%) from ambulatory clinics and 1 from a non-direct patient care position. None of the community pharmacists surveyed had ever made a suspected ADR report in Qatar. Reporters mostly submitted

Table 1 Demographic characteristics of pharmacists responding to the survey of adverse drug reporting (n = 116)

Variable	Value	
	Mean (SD)	
Age (years)	36.2 (8.3)	
	No.	%
Sex (female)	61	52.6
Country of origin (n = 114)^a		
Qatar	4	3.4
Other GCC country	1	0.9
Egypt	40	34.5
Jordan	13	11.2
Other Middle Eastern country	13	11.2
Sudan	21	18.1
Other African country	3	2.6
India/Pakistan	8	6.9
Philippines	5	4.3
Canada/United States	5	4.3
United Kingdom	1	0.9
Highest pharmacy degree		
Bachelors	102	87.9
Masters	9	7.8
Doctorate (PhD or PharmD)	5	4.3
Year of highest pharmacy degree		
2000–11	62	53.4
1990–99	38	32.8
1980–89	12	10.3
1970–79	4	3.4
Country where highest pharmacy degree obtained (n = 109)		
GCC country	2	1.7
Egypt	41	35.3
Jordan	20	17.2
Other Middle Eastern country	7	6.0
Sudan	12	10.3
Other African country	2	1.7
India/Pakistan	10	8.6
Philippines	5	4.3
Other European or Asian country	2	1.7
United Kingdom	6	5.2
Canada/United States	2	1.7
Duration of working as a pharmacist (years) (n = 115)		
< 2	6	5.2
2–5	14	12.1
6–10	41	35.3
11–15	26	22.4
> 15	28	24.1

their documentation to their hospitals (97.0%), but also directly to the SCH (14.7%) or drug manufacturer (5.9%); 18 (52.9%) described receiving some form of acknowledgement for their submission. When asked to describe the ultimate fate of a submitted suspected ADR report in Qatar, over half of all surveyed pharmacists (54.3%) were unsure.

Attitudes and barriers to ADR reporting

Respondents uniformly agreed with the aims of pharmacovigilance activity to promote new understanding of medication; patient safety; and transparency of reporting. A high proportion (84.4%) felt that suspected ADR reporting was a professional obligation and if faced with a patient experiencing a serious ADR, the majority (90.5%) thought they would initiate a suspected ADR report.

Although many respondents agreed that lack of access to a reporting form and remuneration were problematic, a larger proportion disagreed that these issues were barriers. Time constraints were also rated low (21.2%) as a potential impediment. Inability to recognize a suspected ADR was a barrier stated by 39.4% of respondents. Pharmacists identified an increased likelihood of reporting a suspected ADR if the reactions were: serious for the patient (96.2%); novel (90.2%) or associated with a new medication (88.8%); and if some acknowledgment was offered (75.2%). Many respondents (81.6%) felt more pharmacovigilance training and an ability to submit online (68.6%) would facilitate reporting.

Factors influencing ADR reporting

There were no significant differences among respondents when stratified according to sex, age, practice setting

Table 1 Demographic characteristics of pharmacists responding to the survey of adverse drug reporting (n = 116) (concluded)

Variable	Value	
Duration of practice in Qatar (years) (n = 109)	No.	%
< 2	21	18.1
2–5	27	23.3
6–10	42	36.2
11–15	14	12.1
< 15	12	10.3
Pharmacy practice site		
Community	19	16.4
Ambulatory care (private or public) (n = 5)	16	13.8
Hospital (private or public) (n = 3)	72	64.0
Other	9	7.8

^aExamples of countries represented in the categories include: GCC (Oman, Kuwait); other Middle Eastern (Lebanon, Palestine, Syrian Arab Republic); other African (Nigeria, South Africa).
GCC = Gulf Cooperation Council; SD = standard deviation.

and years in practice (Table 2). Only availability of an ADR form was considered a greater barrier for ambulatory care pharmacists when compared with their hospital-based colleagues (11.4% versus 40.3%, $P = 0.002$). When controlling for all other factors in the model, respondents working in hospital settings were over 7 times more likely to have reported a suspected ADR in Qatar.

Discussion

This is the first study evaluating suspected ADR reporting among pharmacists in Qatar. Knowledge of ADR classification was assessed, as it follows that poor knowledge would lead to low declared reporting rates. Correct identification of ADRs through recognition of definitions and patient descriptions was high in our sample and

greater than that reported elsewhere recently [2,5]. Respondents illustrated a good understanding of purpose and positive attitudes towards suspected ADR reporting by pharmacists as the majority considered it a professional obligation.

One-third of respondents had submitted a suspected ADR report in Qatar. This rate is higher than in community pharmacist populations documented recently in the region (approximately 10% in Saudi Arabia, 21% in Turkey), but within reported ranges when surveys among hospital pharmacists in the past decade are considered [2,6]. Hospital pharmacists were most likely to have made a suspected ADR report and this is consistent with studies conducted elsewhere. Factors for such inpatient site-related differences in reporting have been previously proposed and include greater familiarity with pharmacovigilance; constant contact with patients experiencing serious ADRs; and close relationships with physicians who may delegate reporting of ADRs. When controlling for other variables in our model, increased age was also

Table 2 Logistic regression analysis of influence of personal and professional characteristics on adverse drug reporting (ADR) reporting by pharmacists in Qatar

Characteristic	Ever reported ADR in Qatar		Crude analysis		Adjusted analysis ^a	
	No	Yes	OR (95% CI)	P-value	OR (95% CI)	P-value
Sex						
Male	34	20	1.00			
Female	47	14	0.51 (0.23–1.14)	0.100	0.33 (0.11–0.95)	0.04
Age (years)^b			1.01 (0.96–1.06)	0.653	0.86 (0.76–0.99)	0.03
Practice site						
Outpatient	31	4	1.00			
Inpatient	43	29	5.23 (1.67–16.4)	0.002	7.42 (1.90–27.8)	0.003
Duration of practice in Qatar (years)						
< 2	19	2	1.00			
2–5	22	5	2.15 (0.38–12.4)	0.390	1.43 (0.22–9.40)	0.790
6–10	11	3	6.46 (1.20–21.4)	0.020	11.2 (1.60–77.6)	0.020
11–14	25	17	2.59 (0.37–17.9)	0.340	6.78 (0.61–75.7)	0.12
> 15	5	7	13.3 (3.50–84.9)	0.006	23.7 (6.70–83.8)	0.003

^aAdjusted for the effects of the other variables in the table; ^bIn the adjusted analysis, OR of 0.86 indicates that for each additional year of age, a respondent was 0.86 times less likely to reported a suspected ADR in Qatar, controlling for other factors in the model.
OR = odds ratio; CI = confidence interval.

associated with decreased reporting. Older pharmacists in Qatar may have graduated from product-centred education models historically offered in the Middle East region as opposed to more contemporary patient-oriented programmes and, despite greater practical experience, have less clinical confidence in detecting potential ADRs [8]. However, pharmacists with longer practice history in the country in theory have had greater opportunities to encounter, detect and report suspected ADRs in Qatar.

Unavailability of a reporting form has been a stated constraint to voluntary participation in pharmacovigilance activities in other studies [11–13], but this was not a collective barrier in our population; this may be due to the large number of hospital practitioners responding who may have a standard form in place at their site. Pharmacists preferred a single and accessible suspected ADR reporting form with web-based submission capability. Qatar pharmacists did express sentiments similar to both community and hospital pharmacists elsewhere who were unsure if a patient reaction was truly an ADR [14]. Communication and education from regulatory and health professional bodies should emphasize that clinical certainty is not a prerequisite for report submission, as causality assessment can be performed by the pharmacovigilance authority according to documentation of the suspected ADR provided by the reporter.

Uncertainty exists about how submitted suspected ADR reports are handled in Qatar. There is no directive in which reports are automatically advanced to the SCH from patient care sites and there is no indication that reports received by the SCH are consistently or systematically addressed. Local (hospitals, primary-care centres) and national (SCH) bodies alike could enhance pharmacovigilance awareness and reporting with implementation of a feedback mechanism; only half of our respondents described receiving some form of acknowledgement for their submission [15].

There were a number of limitations to our survey warranting discussion. Survey completion was by an internet-based questionnaire. Community pharmacies in Qatar do not generally have computers and so pharmacists with limited or no internet access at home may have been disadvantaged. Non-response error compromises the accuracy of our conclusions and may further contribute to selection bias and restrict the generalizability of our study findings. Those who did not participate in the study may have had less pharmacovigilance awareness; therefore our findings regarding knowledge and attitude may be overestimations and the barriers to reporting underestimations. Finally, because it is not possible to access the identity of pharmacists who have made suspected ADR submissions in Qatar, a case–control

study methodology to assess the factors associated with ADR reporting was not possible. As our study relies on self-reporting, we cannot confirm pharmacists' declared pharmacovigilance activities.

Conclusions

The results indicated that pharmacists' workplaces exerted a strong influence on the reporting of suspected ADRs in Qatar. Most responding pharmacists had never submitted a report in the country, although they expressed positive attitudes towards pharmacovigilance activity and good knowledge of its purpose.

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Isolation and identification of *Legionella pneumophila* from drinking water in Basra governorate, Iraq

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استفراء الفيلقية المُستَروحة والتعرف عليها في مياه الشرب في محافظة البصرة، العراق

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الخلاصة: أجرى الباحثون هذه الدراسة في العراق للتعرف على معدل وجود الفيلقية المُستَروحة في مصادر مختلفة لمياه الشرب في محافظة البصرة، ومدى استجابة مختلف المستفراءات لعدد من المضادات الحيوية. وقد بلغ عدد عينات المياه 222 عينة جمعت في الفترة 2008 – 2009، وكان فيها 49 عينة من محطات تصفية المياه (في نقاط الدخول، وفي صهاريج الترسيب وفي صهاريج الترشيع وفي نقاط الخروج)، و127 عينة من مياه الصنابير، و46 عينة من مياه الإمدادات للصهاريج والمشاريع جمعت من خلال التناضح العكسي. وقد أكدت النتائج وجود الفيلقية المستروحة في مصادر المياه الخام، وفي إمدادات مياه الشرب العامة وفي صهاريج مياه الشرب. ومن بين 258 مستفردة، كان 77.1٪ منها من النمط المصلي 1 وكان 22.9٪ من الأنماط المصلية 2 – 15. وقد أظهرت جميع المستفراءات مقاومة للأدوية، ولا سيما الأميسيلين، إلا أنها تستجيب للدوكسي سيكلين مئة بالمئة. وتدلل الدراسة على أن معدلات انتشار الفيلقيات المستروحة، ولا سيما المجموعة المصلية 1 مؤشر قوي على عدم ملائمة مياه الشرب وعلى ضرورة اتخاذ إجراء عاجل.

ABSTRACT This study in Iraq investigated the occurrence of *Legionella pneumophila* in different drinking-water sources in Basra governorate as well as the susceptibility of isolates to several antibiotics. A total of 222 water samples were collected in 2008–2009: 49 samples from water purification plants (at entry points, from precipitation tanks, from filtration tanks and at exit points), 127 samples of tap water; and 46 samples from tankers and plants supplying water by reverse osmosis. The findings confirmed the presence of *L. pneumophila* in sources of crude water, in general drinking water supplies and drinking water tankers. Of 258 isolates 77.1% were serotype 1 and 22.9% serotypes 2–15. All examined isolates displayed drug resistance, particularly to ampicillin, but were 100% susceptible to doxycycline. The prevalence of *L. pneumophila*, especially serogroup 1, is a strong indicator of unsuitability of drinking water and requires appropriate action.

Isolement et identification de *Legionella pneumophila* dans l'eau potable dans le gouvernorat de Bassora (Iraq)

RÉSUMÉ Une étude en Iraq visait à évaluer l'occurrence de *Legionella pneumophila* dans différentes sources d'eau potable dans le gouvernorat de Bassora ainsi que la sensibilité des isolats à plusieurs antibiotiques. Au total, 222 échantillons d'eau ont été prélevés en 2008 et 2009 : 49 échantillons de stations d'épuration des eaux usées (aux points d'entrée, dans les cuves de précipitation, dans les cuves de filtration et aux points de sortie), 127 échantillons d'eau du robinet et 46 échantillons d'eau de camions-citernes et d'établissements fournissant de l'eau par osmose inverse. Les résultats ont confirmé la présence de *L. pneumophila* dans les sources d'eau brute, dans les sources d'approvisionnement générales et les camions-citernes d'eau de boisson. Sur 258 isolats, 77,1 % étaient de sérotype 1 et 22,9 % de sérotypes 2–15. Tous les isolats examinés étaient pharmacorésistants, en particulier à l'ampicilline, mais 100 % étaient sensibles à la doxycycline. La prévalence de *L. pneumophila*, notamment du sérotype 1, est un puissant indicateur du caractère impropre à la consommation de l'eau de boisson et appelle des mesures adéquates.

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Introduction

The *Legionella pneumophila* species of bacteria comprises over 15 serogroups [1], of which serogroup 1 is responsible for the majority of human infections [2]. Two clinical manifestations have been defined within this spectrum: Legionnaires' disease, which is a pneumonic illness caused by an acute bacterial infection of the lower respiratory tract; and Pontiac fever, which is an influenza-like illness [3]. This Gram-negative bacterium survives in water systems as a parasite of protozoa [4], which are readily found in cooling towers, hot-water distribution systems, bathrooms, swimming pools and fountains [5,6]. Infection results when *L. pneumophila* are transmitted from an environmental source (water or soil) to a host via the inhalation of contaminated aerosols. However, there have been no reports of human-to-human transmission [1]. Therefore, studies concerning the presence of these organisms in drinking-water distribution systems are very important to ensure the good quality of public water.

The present study in Iraq aimed to investigate the occurrence of *L. pneumophila* in different drinking-water sources in Basra governorate (water sanitation plants, drinking water from different districts and reverse-osmosis water-supply plants), as well as the susceptibility of isolates to several antibiotics.

Method

Water samples

A total of 222 water samples were collected in Basra governorate during the period from August 2008 to April 2009. These included: 49 samples from all 13 water purification plants in the governorate (13 samples from entry points, 13 from precipitation tanks, 10 from filtration tanks and 13 samples from exit points); 127 samples of

tap water collected from 18 districts; and 46 water samples collected from reverse-osmosis water suppliers (from tankers supplying water by reverse osmosis in 19 different places and from 5 water-supply plants).

The samples were collected according to *Standard methods for examination of water and wastewater* [7] into sterile sampling bottles, with 10 mL of a sodium thiosulphate solution at 1% in order to neutralize any residual chlorine. The water samples were directly placed in ice, for transportation and examination within the same day. The concentration of residual chlorine for each sample was measured using a chlorine meter (Lovibond 2000) at the time of collection.

Isolation

A duplicate of 5 mL of each sample from water purification plants and tap water and 100 mL of water samples from reverse-osmosis plants and tankers was filtered by the membrane filtration technique using 47 mm cellulose acetate membrane filters with a nominal pore size of 0.45 µm (Sartorius). The membrane filter papers were placed on m-FC agar and incubated in a water bath at 44.5 °C for 24 h and on Legionella agar base (LAB) medium [8] containing *Legionella* growth supplement and *Legionella*-selective supplement which contained dyes, colistin sulphate, vancomycin, trimethoprim and amphotericin B (Himedia). They were incubated at 35 °C in an incubator with humidified atmosphere for 24–72 h.

Identification

Suspected colonies were subcultured in parallel onto LAB medium and were subjected to Gram stain, oxidase, catalase, nitrate reduction, motility, gelatin liquefaction, urease and hippurate test. In addition the slide-agglutination test [1] (HiLegionella latex kit, HiMedia) was used for confirmatory identification of *L. pneumophila* to serogroup 1 and serogroups 2–15.

Antimicrobial susceptibility testing

Isolates were tested for antimicrobial susceptibility by the Stoke disk diffusion method [9] using Mueller–Hinton agar and antibiotic disks (Bioanalyse). The following disks were used: doxycycline (30 µg), erythromycin (15 µg), streptomycin (10 µg), gentamicin (10 µg), chloramphenicol (30 µg) and ampicillin (10 µg). The plates were incubated at 37 °C overnight. The diameter of zone of inhibition of each antimicrobial agent was measured and recorded as resistant, sensitive or intermediate according to the manufacturer's table.

Results

Purification plant samples

The logarithmic numbers of *L. pneumophila* and faecal coliforms from the 13 water purification plants in Basra governorate are shown in Figure 1. All stations (except 1) showed the presence of both *L. pneumophila* and faecal coliforms in raw water. There was no obvious reduction of these 2 groups in precipitation and filtration tanks. Few stations, (5/13) showed the presence of *L. pneumophila*, whereas 9/13 were positive for faecal coliforms in water coming from treatment plants. In precipitation tanks 3/13 stations showed higher number of *L. Pneumophila*, while in the filtration tanks 2/13 stations showed higher numbers of *L. pneumophila*.

Of the 106 isolates recovered from purification plant samples on LAB medium, 55 of them belonged to *L. pneumophila* serogroup 1 while the rest belonged to *L. pneumophila* serogroups 2–15.

Tap water samples

Table 1 shows the average of the logarithmic numbers of *L. pneumophila* and faecal coliforms and the concentration of residual chlorine for the 127 samples of drinking tap water collected from 18 districts.

Table 1 Average residual chlorine concentration and average frequency of *Legionella pneumophila* and faecal coliforms isolated from drinking water (tap water) in Basra governorate, Iraq

District	No. of samples	Average of residual chlorine concentration (mg/L)	Average log no. of faecal coliforms	Average of log no. of <i>L. pneumophila</i>	No. of <i>L. pneumophila</i> isolates
Old Basra	9	1.03	2.89	1.66	10
Al-Jame'ayat	3	0.05	3.03	1.84	5
Al-Ashar	17	0.04	3.44	1.72	18
Al-Ma'aqal	13	0.17	3.43	1.71	15
Al-Hakeemya	8	0.63	2.91	1.50	5
Shatt-Al-Arab	10	0.44	2.66	2.14	8
Al-Esmae'e	5	0	2.32	1.30	4
Al-Hussain	8	1.01	2.78	1.81	8
Al-Tuwaisa	6	0	3.18	2.61	8
Al-Hadi	4	0	3.60	1.44	6
Al-Abela	3	0	3.44	1.54	7
Al-Jubaila	5	1.4	4.00	1.54	7
Al-Jazae'er	3	0	2.87	1.30	5
Al-Jumhurya	3	0	2.33	89.1	3
Al-Junaina	5	0.02	3.72	1.57	5
Al-Mowafakai	5	0	2.66	87.1	5
Al-Fayhaa	9	0.31	2.32	2.31	8
Abu-Al-Khaseeb	11	1.32	2.34	1.60	12
Total	127	–	–	–	139

A total of 133 isolates of *L. pneumophila* serogroup 1 were isolated from these districts, while only 6 isolates of serogroups 2–15 were isolated from Al-Jubaila and Al-Junaina districts. All stations were positive for *L. pneumophila* at frequencies much higher than those recorded for the water coming from treatment plants.

Reverse-osmosis water samples

A total of 41 samples were collected from reverse-osmosis water-supply tankers in 19 different districts in Basra governorate. The average of logarithmic numbers of *L. pneumophila* and faecal coliforms indicated the presence of *L. pneumophila* in 6/19 stations while faecal coliforms was recorded in 12/19 stations. Only 8 isolates of *L. pneumophila* serogroup 1 were isolated from reverse-osmosis water tankers.

In addition 5 main reverse-osmosis plants in Basra governorate were tested for *L. pneumophila*, indicating

the presence of *L. pneumophila* in only 1/5 stations as compared to 3/5 stations harbouring faecal coliforms. Only 3 isolates of *L. pneumophila* serogroup 1 were isolated from the reverse-osmosis plants of the General Company of Petrochemical Industries.

Serogroups

The total number of *L. pneumophila* isolated on LAB medium were 258; serogroup 1 (199 isolates) comprised 77.1% of total isolates and serogroups 2–15 (59 isolates), comprised 22.9% of total isolates.

Antibiotic susceptibility tests

Antibiotic susceptibility testing was done for 10 *L. pneumophila* isolates, 8 isolates belonging to serogroup 1, and 2 isolates belonging to serogroups 2–15. Among serogroup 1 isolates there was 83.0% resistance to ampicillin, 37.5% to erythromycin and 50.0% to chloramphenicol and gentamicin, but 100% sensitivity to doxycycline. On

other hand, isolates of serogroups 2–15 showed 75.0% resistance to ampicillin, 100% intermediate sensitive to erythromycin and streptomycin, 50% sensitive to chloramphenicol and gentamicin and 100% sensitive to doxycycline.

Discussion

Water is a fundamental need for all forms of life, yet human beings continue to pollute the reserves which still remain, thus increasing the risk of diseases that can jeopardize the population [10]. In this study using LAB medium as a selective medium for isolating *L. pneumophila*, most of the water samples in Basra governorate were found to be positive for growth of *L. pneumophila*. These bacteria were isolated from raw water entering the plants and their numbers were uncountable in some plants, confirming that water is a natural reservoir for *Legionella* spp. The bacterium is ubiquitous in fresh water

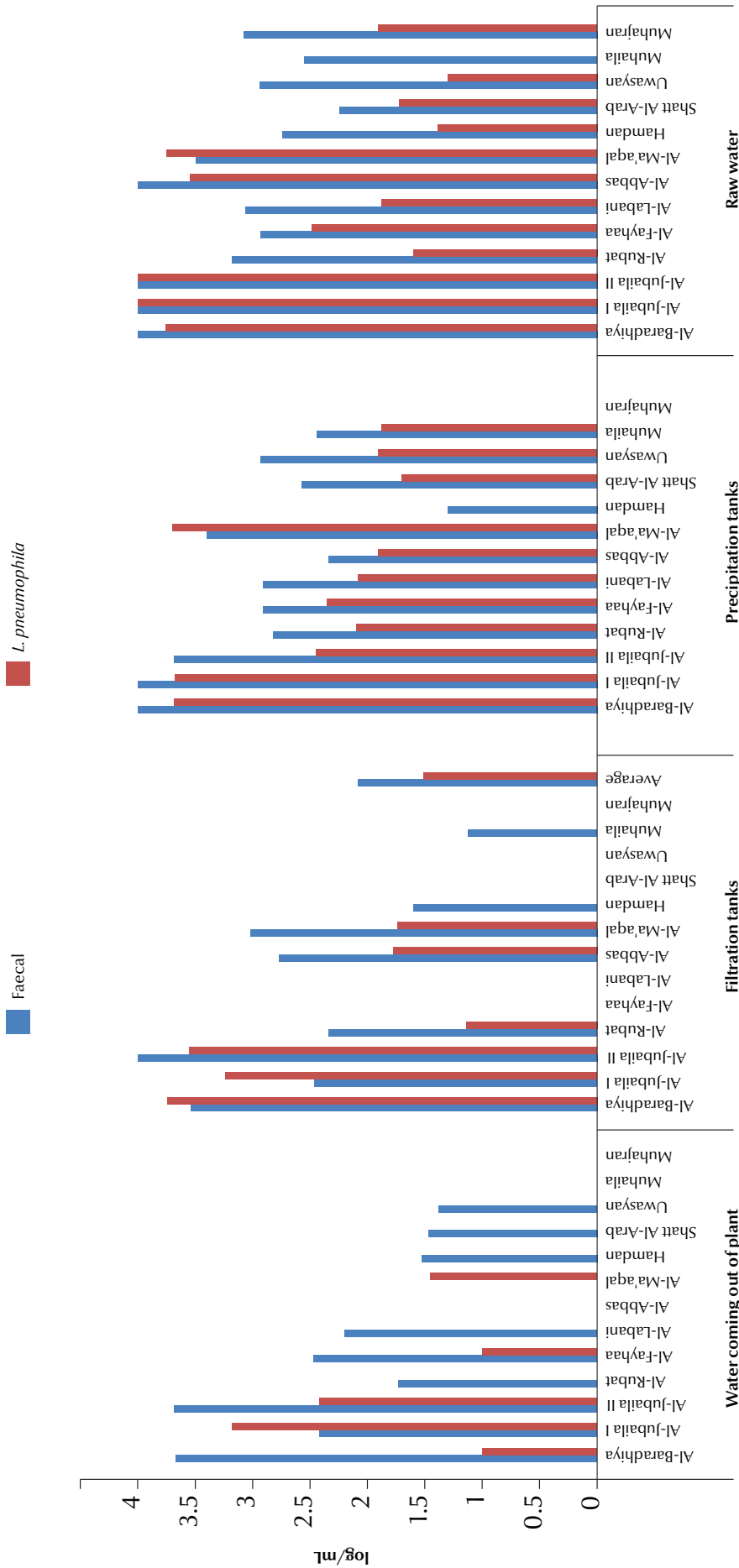


Figure 1 Average of logarithmic numbers per mL of *Legionella pneumophila* and fecal coliforms isolated from water treatment plants in Basra governorate, Iraq

sources [11], which may be due to the inadequacy of sewage water processing before it is dumped into rivers. This bacterium is also able to infect protozoa, a relationship that provides protection for the bacterium against adverse environmental conditions [12], in addition to the presence of organic materials that provide nutrients for *Legionella* spp. growth. This study was similar to that of Wullings and van der Kooij who used culture methods and polymerase chain reaction techniques [13].

L. pneumophila were also isolated from precipitation tanks and it was noted that in some plants the numbers were higher than the numbers in raw water. This may be a result of inefficiency in the primary treating stage of the raw water entering the plants, suggesting that the precipitation tanks work as a reservoir for the growth of these bacteria, perhaps due to the presence of suitable conditions such as precipitants and growth of algae.

L. pneumophila were also present in the filtration units in some of the purification plant samples in Basra and this could be ascribed to the fact that some of the plants are old and/or the filters used in these units are old and there is no maintenance or periodic cleaning or changing of filters. It was observed that in other water treatment plants these bacterium were not detected which provides evidence for the efficiency of filtration units in some cases, which is in agreement with the findings of Bomo et al. [14]. The high growth in the filtration stage of water treatment plants is known to occur in areas of slow-moving water, which may allow growth-supporting materials to accumulate. Passage of water through the rapid sand-filters of the plant almost completely reduces the potential for growth of bacteria, due to removal of growth-enhancing factors and reducing the residence time of bacteria; these findings are similar to the observations of Hoekstra et al. on water passing through rapid and slow sand filters [15].

For the samples of water emerging from plants, it was noted that *L. pneumophila* was absent in most plants and the number of isolates varied between plants, which may be due to the differences in remaining chlorine concentrations in the emission water, as chlorine activity depends on factors such as temperature and pH [2]; these results are compatible with Hsu et al. [16]. *L. pneumophila* is more resistant than other organisms to common standard disinfecting methods. It may, therefore, be found even in disinfected waters with residual chlorine content [17]. A decrease in, or even absence of, chlorine at the extreme ends of the water distribution system increases the risk of growth of the bacterium.

L. pneumophila was isolated from drinking (tap) water in different percentages from district to district and in different areas in the same district of the governorate. The difference in the numbers of isolates across different districts may be due to differences in the biological membranes (biofilms) formed in the distribution pipe networks [18]. Biofilms are essential for growth and proliferation of this bacterium [19]. The combination of organic elements, inorganic elements and the right water temperature create a good environment for *L. pneumophila* proliferation [20,21]. Several studies have indicated that the type of materials of water supply systems (rubber, stainless steel or polyvinyl chloride) affects the formation biofilms [22]. It is well known that Iraq suffers from chronic water deficiency and therefore water treatment plants may not operate in a continuous manner. This creates conditions that contribute to the deterioration of water quality, mainly due to the precipitation and regrowth of pollutants in network pipes, as most of the networks undergo continuous breakage and corrosion that facilitates the entry of pollutants from rain water or infiltration of sewage water into networks. These results are similar to data reported in the literature,

in which *L. pneumophila* was the predominant species in freshwater and municipal drinking water supplies [23] and *L. pneumophila* serogroup 1 was isolated at high frequencies in buildings [24].

Regarding samples of water treated by reverse osmosis it was found that 20 samples were positive for *L. pneumophila*, which is compatible with what Goutziana et al. have observed [25]. Sunlight, temperature, pH and biofilms are factors that affect bacterial activity [13]. This is in addition to the risk of pollution of water during transportation and storage, due to inadequate cleaning and drying practices which provide suitable conditions for the growth and reproduction of pollutants in the stored water.

No association was observed between *L. pneumophila* and the presence of faecal coliforms in our study as *L. pneumophila* were detected in water samples in the absence of faecal coliforms. This opportunistic pathogen has commonly been isolated in the absence of faecal contamination [26].

Development of resistance to antibiotics may be due to increasing use of antibiotics for medical and agricultural purposes and there has been a rise in resistance to these drugs [27]. In the present study isolates were more resistant to ampicillin and less resistant to erythromycin, chloramphenicol and gentamicin. Resistance to ampicillin has been reported previously among *Legionella* spp., due to beta-lactamase production [28]. Erythromycin has usually been considered the antibiotic of choice for the treatment of Legionnaires' disease, but newer antibiotics that are more potent and less toxic are now replacing it [29]. For aminoglycosides such as gentamicin, the mechanism of resistance that is predominantly observed clinically is chemical alteration of the drug catalysed by aminoglycoside-modifying enzymes [30,31]. In this study as all examined isolates were sensitive to

doxycycline, confirming the efficacy of doxycycline on *L. pneumophila* isolates as demonstrated in 7 European countries [32].

This is the first report of *L. pneumophila* in water samples including

water sanitation plants, reverse-osmosis water supplies and samples of tap water from different districts of Basra city, Iraq. The findings confirmed the presence of *L. pneumophila* in crude water sources and in general drinking water

supplies and tankers of drinking water. The prevalence of *L. pneumophila*, especially serogroup 1, is a strong indicator of unsuitability of drinking water and requires appropriate action.

Competing interests: None declared.

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Molecular typing of *Mycobacterium* spp. isolates from Yemeni tuberculosis patients

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تحديد الأنماط الجزيئية للمستفردات من أنواع المتفطرات لدى مرضى السل في اليمن أنس أحمد المحبشي، معاوية مختار، الشيخ محجوب

الخلاصة: أجرى الباحثون هذه الدراسة للتعرف على أنواع المتفطرات المستفردة من مرضى السل الرئوي في اليمن، وقد جمع الباحثون عينات البلغم في الصباح الباكر من 170 مريضاً تم تحويلهم إلى المعهد الوطني للسل في صنعاء للاشتباه بإصابتهم بالسل الرئوي، وقد تم تلوين العينات بملون تسيل نيلسون وزرعت في مستنبت أوغاوا ولفينشتين - جنسن، وتم تضخيم المتتاليات المستهدفة في الجين *rpoB* باستخدام مواد تسبب الطفرة للبادئات للأمام وللخلف، ثم تلا ذلك استخدام الإنزيم *HindIII* للهضم. ومن بين 120 مستفردة حللها الباحثون تبين أن 118 (98.3%) قد تم التعرف عليها على أنها معقد المتفطرة السلية، وأن 2 منها (1.7%) تم التعرف عليهما على أنها متفطرتان غير سليتين. وتوضح النتائج التي توصل إليها الباحثون أن هاتين المستفردتين هما من المتفطرات المقاومة للأدوية، كما أن تحليل متتاليات الدنا أظهر أن تراصف الحمض النووي في الدنا في المستفردات من المتفطرات غير السلية كان يختلف عما هو عليه في معقد المتفطرات السلية.

ABSTRACT This study was done to characterize at the species level *Mycobacterium* spp. isolates from Yemeni pulmonary tuberculosis patients. Early-morning sputum samples were collected from 170 patients referred to the National Tuberculosis Institute in Sana'a city with suspected pulmonary tuberculosis. Samples were processed with Ziehl-Neelsen stain and cultured in Ogawa and Lowenstein-Jensen media. The *rpoB* gene target sequence was amplified using mutagenesis forward and reverse primers followed by *HindIII* enzyme digestion. Of the 120 isolates analysed, 118 (98.3%) were identified as *M. tuberculosis* complex and 2 (1.7%) were identified as mycobacteria other than *M. tuberculosis*. The results showed that those 2 isolates were multi-drug resistant and the DNA sequencing analysis showed that the alignment of nucleic acid of DNA in isolates of mycobacteria other than *M. tuberculosis* was different from that of *M. tuberculosis* complex.

Typage moléculaire des isolats de *Mycobacterium* spp. prélevés chez des patients yéménites atteints de tuberculose

RÉSUMÉ La présente étude a été menée afin de caractériser l'espèce des isolats de *Mycobacterium* spp. prélevés chez des patients yéménites atteints de tuberculose. Des échantillons d'expectoration ont été prélevés tôt le matin chez 170 patients qui avaient été orientés vers l'Institut national de la tuberculose de la ville de Sanaa pour suspicion de tuberculose pulmonaire. Les échantillons ont été traités par coloration de Ziehl-Neelsen et mis en culture sur milieux Löwenstein-Jensen et Ogawa. La séquence cible du gène *rpoB* a été amplifiée selon la méthode des amorces mutagènes directe et inverse suivie par une digestion par l'enzyme *HindIII*. Sur les 120 isolats analysés, 118 (98,3 %) ont été identifiés comme appartenant au complexe *M. tuberculosis* et 2 (1,7 %) comme étant des mycobactéries d'un autre type que *M. tuberculosis*. Nos résultats ont révélé que ces deux isolats étaient pharmacorésistants tandis que l'analyse des séquences d'ADN a montré que l'alignement d'acide nucléique dans les isolats des mycobactéries d'un autre type que *M. tuberculosis* était différent de celui du complexe *M. tuberculosis*.

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Introduction

Tuberculosis (TB) is a disease of major public health concern worldwide. It is a bacterial infectious disease that is considered the second most important cause of death due to an identifiable infectious agent [1]. Approximately one-third of the world's population is infected with latent TB and 5%–10% of this population will develop active stages of the disease during their life time [2].

TB is a highly transmissible disease and infection can occur via inhalation of droplet particles aerosolized from persons infected with *Mycobacterium tuberculosis* or by consumption of milk infected with bovine *M. bovis*. The disease can infect humans and animals, with outcomes ranging from localized lesions to disseminated disease. The genus *Mycobacterium* comprises more than 70 species, some of which are potentially pathogenic to humans and animals and some of which are saprophytic. *Mycobacteria* that cause TB in mammals form the *Mycobacterium tuberculosis* complex (MTC) and include *M. tuberculosis*, *M. africanum*, *M. bovis* or *M. bovis* BCG, *M. microti* and *M. canetti*. Other forms of *mycobacteria* that are considered opportunistic are termed *mycobacteria* other than *Mycobacterium tuberculosis* (MOTT) [3].

Yemen is one of the poorest of the world's low-income countries and TB is one of the most infectious diseases that are endemic in the Yemeni population. The absolute number of TB cases in Yemen is not known, but 37 000 cases were recorded as under treatment throughout the country in the year 2002 [4]. The main objective of this study was to use molecular techniques to identify and characterize *Mycobacterium* spp. isolated from pulmonary TB patients in Yemen.

Methods

Sample collection

The study was conducted on patients referred to the National Tuberculosis Institute in Sana'a city with suspected pulmonary TB based on their presentation with cough more than 2 weeks. The Institute is a specialist referral centre for TB diagnosis and therapy and is situated in Sana'a the capital city of Yemen. The Institute receives TB patients from all regions of Yemen and provides free treatment.

An early-morning sputum sample was collected from 170 patients into wide-mouthed plastic containers. Baseline data of the patients was collected by completion of a questionnaire administered during the collection of samples. Samples were collected between January 2004 and October 2005 and the study was completed in 2008.

The study was approved by the University of Sana'a ethics committee and consent was obtained from the participants before their enrolment in the study.

Laboratory methods

Antibiotic sensitivity testing, using standard methods, was carried out on cultures from all 170 samples. For cost reasons, PCR was done on only 120 of the samples.

Staining and culture methods

Sputum samples were treated with 4% NaOH and stained by Ziehl–Neelsen stain to detect acid-fast bacilli [5]. The sputum samples were cultured on a special egg-based solid medium (Ogawa medium) according to the procedures of the Japan International Cooperation Agency [6]. Typically growth of *Mycobacteria* spp. appears within 3–4 weeks. The colonies are buff in colour with a dry and friable surface and irregular edges.

DNA extraction

Two colonies were taken from the culture medium and placed in 100 µL of sterile distilled water; 100 µL of phenol-chloroform reagent was added and the mixture was vortexed for about 10 s and heated at 80 °C for 20 min. The mixture was stored at –20 °C in microcentrifuge tubes (free from DNA or RNA) until needed [7].

Polymerase chain reaction technique

Primers specific for the *rpoB* gene, encoding the B-subunit of RNA polymerase (*rpoB* DNA, 342–360 base pairs) was the target region for amplification and identification of *Mycobacterium* spp. [8]. The polymerase chain reaction (PCR) mixture was prepared as follows: distilled water 5.0 µL, sample DNA 4.0 µL, PCR buffer 2.5 µL, PCR MgCl₂ 2.0 µL, PCR dNTP 2.5 µL, primers 3 µL, Tag polymerase 1.0 µL. The PCR mixture was gently mixed and amplified using a thermocycler (Perkin Elmer) adjusted to the cycling programme for 30 cycles. The sequence of *rpoB* primers for the mutagenesis forward primer was 5'-CGACCACTTCGGCAACCG-3' and for the mutagenesis reverse primer was 5'-TCGATCGGGCACATCCGG-3'.

Restriction fragment length polymorphism (RFLP)

Following amplification of the *rpoB* gene the product was subjected to digestion by *Hind*III restriction enzymes (Roche) as follows: 15 µL from PCR product was pipetted into PCR tubes, 2 µL of enzyme was added to the tube, 2 µL of enzyme buffer was also added to the tube, then 1 µL of distilled water was added to the mixture and mixed well [9].

DNA sequencing analysis

PCR-amplified DNA of the drug-resistant isolates was commercially

sequenced by MacroGen Company using the BigDye terminator cycling and universal primers.

Results

The age of the whole sample of 170 patients ranged between 12–70 years old, and the largest proportion was in the age group 20–30 years (Table 1). There were significantly more males (117, 69%) than females (53, 31%) ($P < 0.05$).

After culture and sensitivity testing of isolates, 15 antibiotic resistant isolates were found: 5 (33.3%) were resistant to 1 drug, 4 (26.6%) to 2 drugs, 4 (26.6%) to 3 drugs and 2 (13.3%) to 4 drugs. The resistance data were as follows: isoniazid 14/15 (93%), rifampicin 8/15 (53%), streptomycin 5/15 (33%) and ethambutol 6/15 (40%).

Our results showed that mycobacterial DNA was amplified successfully using the relevant primers and the size of DNA was 360 bp compared with the molecular weight marker (100 bp) (Figure 1).

The PCR-RFLP results showed that 118/120 (98.3%) of the isolates were MTC, whereas 2/120 (1.7%) were MOTT (Figure 2A and B).

The DNA sequencing analysis results showed that the DNA sequence of MTC strains were different from MOTT strains (Figure 3).

Discussion

A recent health report on Arab countries by the World Health Organization declared that TB was an important public health problem in the 19 Arab countries of the Eastern Mediterranean Region, affecting 240 000 people with 53 000 deaths every year; 85% of the deaths occurred in adults [10]. In Yemen, TB is considered one of the major infectious

Table 1 Age and sex distribution of the study patients who were sputum-smear positive for tuberculosis

Variable	No.	%
Age group (years)		
10–< 20	40	24
20–< 30	67	39
30–< 40	27	16
40–< 50	18	11
50–< 60	10	6
> 60	8	5
Sex		
Male	117	69
Female	53	31
Total	170	100

diseases recorded in the national disease list [11]. A rapidly increasing population, poor quality health services, very low annual income of individuals and the whole country's poor economic status are the most important factors responsible for the high incidence of TB in the country [12].

In the present study 170 patients were recruited who were suspected of having pulmonary TB based on their presentation with cough more than 2 weeks. The age of the patients ranged between 12–70 years old, and the

highest prevalence was among the age group 20–30 years. These results are in agreement with previous reports from the national disease surveillance infectious diseases centre and the Ministry of Health [11,12]. As previously reported, in this study males were significantly more affected than females [13]. In Yemen the higher rate of TB in males may be attributed to the different habits of males, especially smoking the waterpipe (*nargile* or *mada'a*), which is usually shared between different persons. Another prevalence study on pulmonary TB

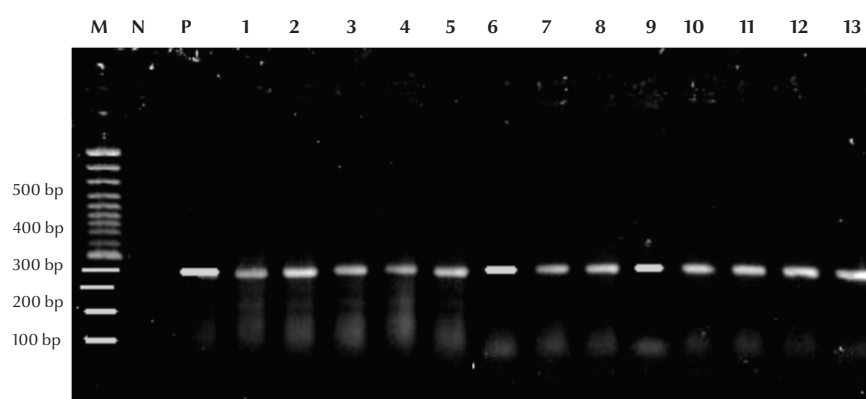


Figure 1 Polymerase chain reaction assay results using the MF and MR primers for detection and amplification of the *Mycobacterium rpoB* gene from the isolates with leader marker (100 bp) to detect the size of amplified DNA
M = molecular weight control marker (100 bp); P = positive control; N = negative control; lanes 1–13 from patient samples

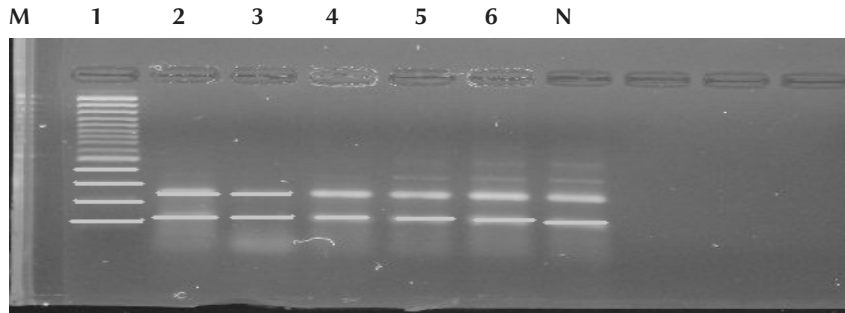


Figure 2A Polymerase chain reaction–restriction fragment length polymorphism assay using restriction enzymes of confirmed tuberculosis patients; lanes 1,2,3,4,5,6 were *Mycobacterium tuberculosis* complex isolates that showed 2 fragments. M = control marker 100 bp; N = negative control

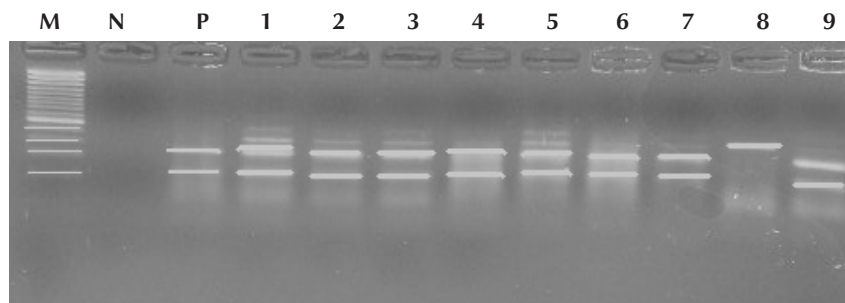


Figure 2B Polymerase chain reaction–restriction fragment length polymorphism assay using restriction enzymes of confirmed tuberculosis patients; lanes 1,2,3,4,5,6,7,9 were *Mycobacterium tuberculosis* complex isolates that showed 2 fragments, whereas lane 8 is *Mycobacteria* other than *M. tuberculosis* that showed 1 fragment. M = control marker 100 bp; N = negative control; P = positive control

- 1- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 2- ACCA-TCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 3- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 4- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 5- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 6- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 7- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 8- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 9- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 10- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 11- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 12- ACCA-GCCAGCTGAGCCAATTCATGGACCAGAAACAACCCGCTGTCGGGGTTGACCCACAA
- 13- ACCGCGTCGTGTATGACTCTGTATACAGAGGAGTACGGCGCGCGTGGTGGTCTCCAT

Figure 3 DNA sequence analysis alignment showing the difference between nucleic acid of *Mycobacterium tuberculosis* complex (samples 1-12) and *Mycobacteria* other than *M. tuberculosis* (sample 13)

attributed the low prevalence of TB among women to underdetection of TB in females because women often choose medical care providers operating outside the national TB control centres [14].

Routine sputum smears and *Mycobacterium* spp. cultures confirmed the presence of acid-fast bacilli in the 170 sputum samples examined. However, acid-fast staining does not identify the *Mycobacterium* to the species level. In addition the time required to detect the organism by routine culture is approximately 4–8 weeks. PCR assay was therefore used to characterize 120 of the isolates. The *rpoB* gene was successfully amplified in all isolates and enabled identification of *Mycobacterium* spp. following restriction of the PCR product by *HindIII* restriction enzyme that produced 2 DNA fragments in the amplicons of *M. tuberculosis*.

The PCR-RFLP results identified 98.3% of isolates as MTC and 1.7% samples as MOTT. Interestingly the MOTT isolates were resistant to isoniazid, rifampicin and streptomycin. MOTT have been reported to cause infection in humans and to complicate treatment regimens since they may not respond to routinely used anti-TB drugs [15–17]. Based on the results of this study we recommend the use of molecular techniques for identification of the *Mycobacterium* spp. before initiation of treatment.

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High prevalence of *Klebsiella pneumoniae* carbapenemase-mediated resistance in *K. pneumoniae* isolates from Egypt

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معدل انتشار مرتفع للمقاومة للكلبيسيلا الرئوية بتواسط إنزيم كارباينيماز في مُستفردات الكلبيسيلا الرئوية في مصر
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الخلاصة: إن البزوغ والانتشار السريع لمستفردات الكلبيسيلا الرئوية المقاومة للمضادات الحيوية والتي تحتوي على الجين bla_{kpc} الذي يرمز لإنتاج إنزيم كارباينيماز قد أدى إلى تعقيد التدبير العلاجي للمرضى المصابين بالعدوى. وقد أجرت البحوثات هذه الدراسة في مستشفى للرعاية الثالثة في مصر باستخدام مقايضة التفاعل السلسلي للبولىميراز في الزمن الحقيقي لاختبار مستفردات الكلبيسيلا الرئوية التي لا تستجيب للإرتابينم لكشف وجود الجين bla_{kpc} ولمقارنة النتائج مع اختبار هودج المعدل. وقد درست البحوثات الاستجابة للمضادات الحيوية بالطرق المعيارية، وقُمن بتفسيرها باتباع نقاط الفصل القديم المعتمدة لدى معهد المعايير السريرية والمختبرية (M100-S19) بالنسبة لمركبات كارباينيم، إلى جانب نقاط الفصل المنقحة (M100-S22). وشملت الدراسة 45 مستفردة غير مزدوجة للكلبيسيلا الرئوية أخذت من عينات سريرية مختلفة، واتضح للبحوثات أن معدل انتشار مرتفعاً للمستفردات غير المستجيبة للإرتابينم (44.4%) قد تم تسجيله باستخدام نقاط الفصل المنخفضة والجديدة المعتمدة لدى معهد المعايير السريرية والمختبرية، كما تأكد للبحوثات وجود الجين bla_{kpc} في 14 من بين 20 مستفردة (70%)، ويعزى ارتفاع معدل انتشار عدم الاستجابة للإرتابينم في مستشفى الرعاية الثالثة في مصر على الأغلب إلى آليات المقاومة التي تتواسطها إنزيمات كارباينيماز في مستفردات الكلبيسلات الرئوية.

ABSTRACT The emergence and rapid spread of antibiotic-resistant *Klebsiella pneumoniae* isolates harbouring the bla_{kpc} gene that encodes for carbapenemase production have complicated the management of patient infections. This study in a tertiary care hospital in Egypt used real-time PCR assay to test ertapenem-nonsusceptible isolates of *K. pneumoniae* for the presence of the bla_{kpc} gene and compared the results with modified Hodge test. Antibiotic sensitivity was performed by standard methods, and interpreted following both the old CLSI breakpoints (M100-S19) for carbapenems and the revised breakpoints (M100-S22). From the 45 non-duplicate isolates of *K. pneumoniae* recovered from different clinical specimens, a high prevalence of ertapenem-nonsusceptible isolates (44.4%) was reported using the new lower CLSI breakpoints. The bla_{kpc} gene was confirmed in 14/20 (70.0%) of these isolates. The high prevalence of ertapenem nonsusceptibility at a tertiary care hospital in Egypt was predominantly attributed to *K. pneumoniae* carbapenemase-mediated resistance mechanisms in *K. pneumoniae* isolates.

Prévalence élevée de la résistance de *Klebsiella pneumoniae* médiée par les carbapénèmases dans des isolats de *K. pneumoniae* en Égypte

RÉSUMÉ L'émergence et la propagation rapide des souches de *Klebsiella pneumoniae* résistantes aux antibiotiques et porteuses du gène bla_{kpc} codant la production de carbapénèmases ont compliqué la prise en charge des infections des patients. La présente étude menée dans un hôpital de soins tertiaires en Égypte a utilisé la méthode de PCR en temps réel pour évaluer la présence du gène bla_{kpc} dans les isolats de *K. pneumoniae* non sensibles à l'ertapénème, puis a comparé les résultats à l'aide du test de Hodge modifié. La sensibilité aux antibiotiques a été évaluée à l'aide des méthodes standards, puis a été interprétée selon les anciens seuils du *Clinical and Laboratory Standards Institute* (M100-S19) pour les carbapénèmes et selon les seuils révisés (M100-S22). Après l'analyse des 45 isolats non-dupliqués de *K. pneumoniae* prélevés à partir de différents échantillons cliniques, une prévalence élevée d'isolats non sensibles à l'ertapénème (44,4 %) a été rapportée selon les nouveaux seuils plus bas du *Clinical and Laboratory Standards Institute*. La présence du gène bla_{kpc} a été confirmée dans 14 isolats sur 20 (70,0 %). La forte prévalence de la non sensibilité à l'ertapénème dans un hôpital de soins tertiaires en Égypte était principalement imputable aux mécanismes de résistance médiés par les carbapénèmases dans les isolats de *K. pneumoniae*.

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Introduction

Klebsiella pneumoniae carbapenemases (KPCs) are Ambler class A plasmid-encoded enzymes that are capable of hydrolyzing all beta-lactam antibiotics, including monobactams, extended-spectrum cephalosporins and carbapenems [1,2]. Originally described in 2001 [3], pathogens harbouring these antibiotic-resistance enzymes have been reported from the United States of America (USA) [4–7], France, China, Sweden, Norway, Colombia, Brazil, Scotland, Germany and Spain [8–11]. Epidemic situations have also been reported in Israel and Greece [12,13]. An important challenge to developing a standardized definition of bacterial isolates resistant to carbapenems is a recent (mid-2010) change in the Clinical and Laboratory Standards Institute (CLSI) interpretative criteria (breakpoints) for determining susceptibility to carbapenems among Enterobacteriaceae [14,15]. These new recommendations lowered the breakpoints and removed the requirement for testing for carbapenemases, e.g. by modified Hodge test (MHT), to determine susceptibility. However, based on clinical and microbiological data, ertapenem breakpoints were modified again in January 2012 (M100-S22) by doubling the dilution (to ≤ 0.5 µg/mL) [16].

In addition to beta-lactam/carbapenem resistance, nonsusceptible organisms can carry genes that confer high levels of resistance to many other antimicrobials, often leaving very limited therapeutic options [17,18]. The *bla*_{KPC} gene encodes for KPC enzyme production. Although carbapenemases have been identified in many species of Enterobacteriaceae, *K. pneumoniae* remains the most common organism carrying resistance-encoding genes [2]. Carbapenem resistance in *K. pneumoniae* may also be due to production of other carbapenemases [19] or to changes in outer membrane porin proteins [20], often combined with

production of an extended-spectrum beta-lactamase, AmpC or both [19,21].

Molecular detection of the *bla*_{KPC} gene by polymerase chain reaction (PCR) assay provides laboratories with a means to quickly identify the presence of this important resistance determinant [22,23]. Considering the demonstrated potential for rapid horizontal and vertical transmission of the *bla*_{KPC} gene, prompt recognition is important to controlling the spread of KPCs. In the present study we describe a real-time PCR assay to detect all variants of the *bla*_{KPC} gene and the use of this assay to test clinical isolates of *K. pneumoniae*. We also tested ertapenem-nonsusceptible isolates using MHT.

Methods

Study isolates

A prospective study was conducted over a period of 6 months (June 2011 to December 2011) at the Suez Canal University hospital, Ismailia, Egypt. A total of 45, single-patient *K. pneumoniae* isolates were included in the study. These isolates were recovered from urine ($n = 13$), blood ($n = 8$), respiratory tract ($n = 12$) and other clinical sites ($n = 12$) from patients admitted to the intensive care unit and different wards of the hospital. Full identification was carried out using the API 20E system (bioMérieux).

Ethical approval to perform the study was obtained from the ethics committee in the Faculty of Medicine, Suez Canal University and the management board of the hospital. All the included patients consented to the collection of specimens before the study was initiated.

Susceptibility testing

Antibiotic susceptibility testing was determined using the modified Kirby-Bauer method following the CLSI guidelines. The following antimicrobial agents were included in the panel:

ampicillin, amoxicillin/clavulanic acid, ceftriaxone, cefepime, cefazolin, ceftioxin, ciprofloxacin, gentamicin, tobramycin, imipenem, ertapenem, meropenem, trimethoprim/sulfamethoxazole, piperacillin, piperacillin/tazobactam and tobramycin (Oxoid).

Isolates were further subjected to minimum inhibitory concentration (MIC) testing for imipenem and meropenem using the Oxoid MIC evaluator strip (Thermo Fisher Scientific) and for ertapenem using the gradient strip E-test (bioMérieux); boxes were allowed to equilibrate at room temperature for at least 1 h before opening. For all isolates the inocula for strip tests were matched to a 0.5 McFarland standard. Results were read in accordance with the manufacturers' directions and interpreted following both the old CLSI M100-S19 breakpoints and the revised breakpoints in the M100-S22 document issued in January 2012 [14–16].

Suspension of a known KPC-producing isolate [*K. pneumoniae* American Type Culture Collection (ATCC) BAA-1705], recovered on the MacConkey agar, was used as quality control strain. A second carbapenem-susceptible *K. pneumoniae* (ATCC 700603) was used as negative control.

Stocks of 20 distinct single-patient *K. pneumoniae* isolates representing different antibiogram patterns and showing MIC ≥ 1 µg/mL ($n = 20$) for ertapenem, using the revised carbapenem breakpoints (M100–S22, January 2010), were tested for carbapenemases by MHT and stored in tryptic soy broth with 20% glycerol at -20°C until further testing by *bla*_{KPC} real-time PCR.

Detection of *bla*_{KPC} by real-time PCR

Fresh, well-isolated test colonies grown on sheep-blood agar plates following overnight incubation were used for DNA extraction using the QIAamp DNA mini kit (Qiagen) according to the manufacturer's protocol. Briefly, a 2.0 McFarland standard bacterial

suspension was prepared in saline, and bacterial DNA was extracted from 200 μL (1.2×10^8 colony forming units) of the suspension. Extracted bacterial DNA was eluted from the columns in 100 μL elution buffer and stored at -20°C .

The TaqMan real-time KPC PCR assay uses previously published primers and probes which detect all currently described KPC variants [24]. The sequences were as follows: for the KPC forward primer, 5'-GCG GAA CCA TTC GCT AAA CTC GAA-3'; for the KPC reverse primer, 5'-AGA AAG CCC TTG AAT GAG CTG CAC-3'; and for the KPC probe, 5'-/6-FAM/ATA CCG GCT CAG GCG CAA CTG TAA GTT A/6-TAMRA/-3' (where 6-FAM represents 6-carboxy-fluorescein and 6-TAMRA represents 6-carboxytetramethylrhodamine).

Real-time PCR was performed with 2 μL template DNA in a total reaction volume of 10 μL containing 1 $\times$ LightCycler FastStart DNA master hybridization probe reagent (Roche Diagnostics), 3.5 mM MgCl_2 , and 2 μM of primers for *bla*_{KPC} and the TaqMan probe.

A negative control consisting of the reaction mixture and water (in place of template DNA) was added in each run. In addition to negative controls, a reference *K. pneumoniae* strain (ATCC BAA-1705) was selected as the positive control.

The LightCycler 2.0 instrument (Roche Diagnostics) was used for the amplification and detection of the *bla*_{KPC}

gene using the following PCR cycling conditions; after an initial denaturation step of 3 min at 95°C , a 2-step PCR procedure was used consisting of 30 s at 95°C and 1 min at 60°C for 45 cycles.

Data were obtained during the annealing period. Fluorescence was measured once every cycle immediately after the 60°C incubation (extension step). Fluorescence curves were analysed with the *LightCycler* software, version 4.0. The results were expressed by determination of the threshold cycle (Ct) value which marked the cycle at which the fluorescence of the sample became significantly different from the baseline signal. A sample was regarded as positive when the *LightCycler* software determined a Ct in the quantification analysis screen.

When analysing the results, it is important to only consider amplification between 10–35 cycles as positive. Amplification prior to 10 cycles means the template should be diluted before repeating. Amplification after 35 cycles can indicate trace contamination. The no template (water) control should not yield a product (Ct > 40). PCR positive isolates with reduced ertapenem MIC were considered to be KPC positive.

Detection of KPC by the MHT

Isolates that were nonsusceptible to ertapenem (i.e. resistant and intermediate isolates) were also tested by the MHT previously described [25]. Briefly, a 0.5 McFarland suspension of *Escherichia coli* (ATCC 25922), was used to prepare a lawn culture on a Mueller–Hinton agar plate (Becton Dickinson), and a 10 μg

ertapenem susceptibility disk (Oxoid) was placed in the centre of the test area. Test isolates were subcultured onto sheep-blood agar plates (Becton Dickinson) to establish pure cultures. The isolate was then streaked in a straight line from the edge of the disk to the edge of the plate and was incubated overnight at 35°C in ambient air. After 24 hours of incubation, the plate was examined for a cloverleaf-shaped indentation at the intersection of the test organism and the *E. coli* ATCC 25922 within the zone of inhibition. The presence of a cloverleaf-shaped indentation was considered MHT positive.

Results

By using current breakpoints (M100-S22) for carbapenem interpretation, 20 out of 45 *K. pneumoniae* isolates (44.4%) were reported as nonsusceptible (intermediate and resistant) to ertapenem (Table 1). However, when the old 2009 breakpoints were used, ertapenem interpretation classified only 15 (33.3%) of isolates as nonsusceptible and 30 (66.7%) as susceptible. Of the 5 isolates that was counted as susceptible by the 2009 guidelines yet nonsusceptible by the new guidelines, 3 isolates were positive for the *bla*_{KPC} gene; these isolates were susceptible to meropenem and imipenem. Among the isolates tested, 40.0% and 37.8% were nonsusceptible to imipenem and meropenem respectively at the new CLSI resistance breakpoint of $\geq 2 \mu\text{g}/\text{mL}$ for both drugs (Table 1).

Table 1 Minimum inhibitory concentration results for carbapenem antibiotics on *Klebsiella pneumoniae* isolates (n = 45) using different Clinical and Laboratory Standards Institute (CLSI) breakpoints

Antibiotic agent	Older breakpoints ^a				Current breakpoints ^b			
	Susceptible		Nonsusceptible ^c		Susceptible		Nonsusceptible ^c	
	No.	%	No.	%	No.	%	No.	%
Imipenem	34	75.6	11	24.4	27	60.0	18	40.0
Meropenem	33	73.3	12	26.7	28	62.2	17	37.8
Ertapenem	30	66.7	15	33.3	25	55.6	20	44.4

^aCLSI M100-S19 criteria [14,15]; ^bCLSI M100-S22 criteria [16]; ^cIntermediate and resistant.

Table 2 Results of modified Hodge test (MHT) and polymerase chain reaction (PCR) assay for *bla*_{KPC} gene on nonsusceptible *Klebsiella pneumoniae* isolates (n = 20)

Modified Hodge test results	Polymerase chain reaction results		Total
	PCR+ve	PCR-ve	
MHT+ve	14	3	17
MHT-ve	0	3	3
Total	14	6	20

+ve = positive; -ve = negative.

Real-time KPC PCR assay results were used to confirm that carbapenem resistance in *K. pneumoniae* isolates was due to production of a KPC. Of the 20 *K. pneumoniae* isolates with reduced susceptibility to ertapenem (defined as ≥ 1 $\mu\text{g/mL}$), according to the revised clinical breakpoints, 14 isolates were found positive for KPCs by MHT and by PCR detection of the *bla*_{KPC} gene (Table 2). Of the remaining 6 isolates that were negative by PCR, 3 isolates were positive by MHT.

Discussion

The emergence and rapid spread of antibiotic-resistant *K. pneumoniae* isolates harbouring the *bla*_{KPC} gene that encodes for carbapenemase production have complicated the management of patients' infections [1,2]. To our knowledge, this is the first published report of KPC-producing *K. pneumoniae* isolated from patients at a tertiary care hospital in Egypt. In our study we used ertapenem to screen for carbapenemases, as ertapenem is the least active carbapenem against KPCs [26] and as the use of this drug in automated or manual susceptibility testing has been found to be a highly sensitive method for the detection of KPCs [26,27]. Despite the limited number of isolates included, we were able to show a high prevalence of ertapenem non-susceptibility, accounting for 44.4% of *K. pneumoniae* isolates tested. This high prevalence reflected the new lower CLSI breakpoints for carbapenems. When the previous CLSI

breakpoints for ertapenem were used (resistant > 4 $\mu\text{g/mL}$; susceptible ≤ 2 $\mu\text{g/mL}$), only 33.3% would be counted as nonsusceptible. A high prevalence of ertapenem resistance was similarly reported by many investigators in different countries [5,19]. For instance, in a study from China none of the 77 clinical isolates collected from 2002 to 2009 were susceptible to ertapenem and only 6.5% and 1.3% of isolates were susceptible to imipenem and meropenem respectively [28].

Of the 5 isolates that were counted as ertapenem-susceptible by the old CLSI M100-S19 breakpoints but non-susceptible by the revised breakpoints, 3 isolates were positive for *bla*_{KPC} genes and MHT, signifying the improved rate of detection of KPC-mediated resistance when using the new CLSI breakpoints. Likewise, the new breakpoints increased the proportion of isolates counted as nonsusceptible to imipenem and meropenem (to 40.0% and 37.8% respectively), although these were less than for ertapenem.

We also described in this study, a real-time PCR designed to detect and characterize genes encoding all KPC variants. Using this assay, we documented for the first time in Egypt the presence of isolates producing KPCs. Isolates with ertapenem MIC ≥ 1 $\mu\text{g/mL}$ were further investigated to determine the prevalence of KPC enzymes. We were able to confirm the presence of *bla*_{KPC} genes in 14 (70.0%) of ertapenem-nonsusceptible isolates, which comprised 31.1% of all isolates tested,

indicating that the increased prevalence of ertapenem non-susceptibility was predominantly attributed to KPC-mediated resistance mechanisms in *K. pneumoniae*. Prevalence rates of KPC-positive *K. pneumoniae* isolates of $> 30\%$ have been recorded in some institutions in the eastern USA, in association with nosocomial outbreaks [27].

Our results suggest performing confirmatory testing for the presence of KPC for all ertapenem-resistant bacteria. All KPC-producing bacteria were also MHT positive, indicating the usefulness of doing this phenotypic testing. However, due to the more rapid turnaround time of PCR assays, this assay might be more suitable as an initial screening test for detecting KPC-mediated carbapenem resistance. On the other hand, PCR is more technically challenging, prone to inhibition and may miss new variants of KPC arising from genetic mutation.

Of the ertapenem-nonsusceptible isolates 6 were negative by real-time *bla*_{KPC} PCR and, of those, 3 isolates were positive by MHT. Two possibilities exist that may explain these 3 MHT-positive/KPC-PCR-negative isolates. First, as reported by Schechner et al. KPC PCR could be falsely negative due to inhibitory substances in the reaction or to technical inexperience of the laboratory [29]. However, the most probable reason could be the presence of other carbapenemases, such as the metallo-beta-lactamases and the member of the *Serratia marcescens* (SME) family of carbapenem-hydrolyzing beta-lactamases, SME-1, which can produce a positive result for MHT but negative for *bla*_{KPC}. So although the new CLSI recommendations lowered the breakpoints of carbapenems and removed the requirement for testing for carbapenemase (e.g. MHT) to determine susceptibility [15], performing MHT as an adjunct to KPC PCR may increase the likelihood of detecting other carbapenemases. Furthermore, the current recommendation is to still

to perform MHT for infection control and epidemiological purposes.

Our study had some limitations. First, the number of isolates included in the study was limited by the low incidence of *K. pneumoniae*-associated infections in our institution during the study; nonetheless, the available results provided robust pilot data. Secondly, molecular detection of *bla*_{KPC} genes was further limited to isolates nonsusceptible to ertapenem. However, this did not substantially compromise our study findings, especially when using

the new lower CLSI breakpoints for interpretation. Thirdly, we did not screen our isolates for other resistance determinants, such as AmpC or outer membrane proteins, owing to limited funding available.

In summary, our data showing an increased prevalence of ertapenem-nonsusceptible *K. pneumoniae* isolates partly reflects lowering of clinical breakpoints but also indicates the spread of carbapenemases, principally KPC types, in Suez Canal University hospital, Egypt. Confirmatory testing for

the presence of KPCs is required for all ertapenem-resistant bacteria. Real-time PCR assay described here provides a useful tool to rapidly and accurately detect *bla*_{KPC}-positive bacteria, which is an important step in controlling their spread.

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Prognostic factors of *Atractylis gummifera* L. poisoning, Morocco

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العوامل الإنذارية في التسمم بالإشخيص (شوك العلك) في المغرب

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الخلاصة: يمثل التسمم بنبات الإشخيص (شوك العلك) في المغرب السبب الرئيسي للتسمم بالنباتات، ولا سيما بين الأطفال. وقد أجرى الباحثون هذه الدراسة التي شملت جميع المصابين بالتسمم الذين راجعوا المركز المغربي لمكافحة التسمم في الفترة من كانون الثاني/يناير 1981 وكانون الأول/ديسمبر 2009، وعددهم 467، وهي دراسة استيعادية للخصائص ولعوامل الخطر للتسمم بالإشخيص. ووجد الباحثون أن أكثر فئات الأعمار تعرضاً للمخاطر هم الأطفال (63.4% من الحالات)، وأن معظم الحالات سببها التعرض العرضي (غير المقصود) (75.5%)، إلا أن بعض الحالات نجمت عن استخدامات بقصد المعالجة (18.1%) أو محاولات الإجهاض (7.4%). وقد راجع المرضى بأعراض وعلامات تسمم معتدلة الشدة (الدرجة 2) في 22.3% من الحالات، وشديدة (الدرجة 3) في 21% من الحالات. وبلغ معدل الوفيات 39.2%، وكانت معظم حالات الوفيات (81.1%) بين الأطفال الذين تقل أعمارهم عن 15 عاماً تلو التعرض العرضي (غير المقصود). وقد اتضح من تحليل التحوف اللوجستي المتعدد المتغيرات أن عوامل اختطار الوفاة هي الغيبوبة (نسبة الأرجحية = 20.5)، والالتهاب الكبدي (نسبة الأرجحية = 52.7)، والسكن في الأرياف (نسبة الأرجحية = 7.26)، في حين كان تنظيف المعدة من العوامل الواقية (نسبة الأرجحية = 0.26).

ABSTRACT In Morocco, acute *Atractylis gummifera* L. poisoning represents the leading cause of death by plant poisoning especially for children. All cases received in the Moroccan poison control centre from January 1981 to December 2009 ($n = 467$) were included in a retrospective study of the characteristics and risk factors of *A. gummifera* L. poisoning. The most vulnerable age group was children (63.4% of cases). Most cases were due to accidental exposure (75.5%), but some were from therapeutic use (18.1%) or attempted abortion (7.4%). Patients presented with moderate poison severity signs (grade 2) in 22.3% of cases or severe signs (grade 3) in 21.0%. The mortality rate was 39.2%. The majority of deaths (81.1%) occurred in children aged < 15 years following accidental exposure. Multivariate logistic regression analysis revealed that risk factors for mortality were coma (OR = 20.5); hepatitis (OR = 52.7) and rural residence (OR = 7.26), while gastric decontamination was a protector factor (OR = 0.26).

Facteurs pronostiques d'intoxication par *Atractylis gummifera* L. au Maroc

RÉSUMÉ Au Maroc, l'intoxication aiguë par *Atractylis gummifera* L. représente la principale cause de décès dus à une intoxication par les plantes, en particulier chez les enfants. Tous les cas reçus au centre antipoison marocain entre janvier 1981 et décembre 2009 ($n = 467$) ont été inclus dans une étude rétrospective des caractéristiques et des facteurs de risque d'une intoxication par *A. gummifera* L. Le groupe d'âge le plus vulnérable était les enfants (63,4 % des cas). La plupart des cas étaient dus à une exposition accidentelle (75,5 %), mais certaines expositions avaient des visées thérapeutiques (18,1 %) ou abortives (7,4 %). Les patients présentaient des signes d'intoxication d'une intensité modérée (grade 2) dans 22,3 % des cas, ou d'une intensité sévère (grade 3) dans 21,0 % des cas. Le taux de mortalité était de 39,2 %. La majorité des décès (81,1 %) se sont produits chez des enfants de moins de 15 ans, à la suite d'une exposition accidentelle. L'analyse de régression logistique multivariée a révélé que les facteurs de risque de mortalité étaient un coma (O.R. = 20,5), une hépatite (O.R. = 52,7) et la résidence en milieu rural (O.R. = 7,26), tandis qu'une décontamination gastrique constituait un facteur protecteur (O.R. = 0,26).

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Introduction

Atractylis gummifera L. also called “glue thistle” or “*addad*” is a poisonous plant widespread in North Africa (Tunisia, Morocco, Algeria), Asia Minor and southern Europe (Spain, Portugal, Italy, Greece), but also in France (Corsica) [1]. This thistle, accidentally ingested or used in traditional medicines, causes serious poisoning incidents with fatal outcome in many cases, and constitutes a public health problem especially for children in the Mediterranean region [2]. Victims of intoxication are mainly rural children, who confuse the root with other edible plants, such as the artichoke *Scolymus hispanicus* L., or use the white substance, which it exudes as chewing gum. Intoxication can also occur during use of the glue thistle as a medicinal plant because of its antipyretic, diuretic, abortifacient, emetic and purgative properties [3].

The toxic effect of this plant arises from 2 diterpenoid toxicants causing toxicity—atractyloside and carboxyatractyloside—which are a powerful mitochondrial inhibitors of oxidative phosphorylation and interact with a mitochondrial protein involved in mitochondrial membrane permeabilization. This action is exerted especially in cells rich in mitochondria such as hepatocytes and in proximal tubular epithelial cells. The consequences are cell necrosis with extensive liver damage and kidney failure. Poisoned patients manifest characteristic symptoms such as nausea, vomiting, epigastric and abdominal pain, diarrhoea, hepatitis, anxiety, headache and convulsions, often followed by coma. No specific pharmacological treatment for *A. gummifera* intoxication is yet available and all the current therapeutic approaches are only symptomatic.

In Morocco, poisoning by this plant is very common and frequently fatal [4,5] and represents the leading cause of death by plant poisoning in Morocco

[6]. The thistle is available in herbal stalls and markets and is frequently found in nature in the wild, except in desert areas or dry lands and the Anti-Atlas mountains [7]. From 1980 to 2008, the poison control centre of Morocco has collected 4287 cases of poisoning by plants, of which death occurred in 7.3%. The glue thistle was implicated in 77.6% of these deaths [6].

Published data about *A. gummifera* L. poisoning are rare and limited to a few clinical cases. A comprehensive study with a determination of risk factors has never been made. The current study aimed to evaluate a series of cases of acute *A. gummifera* L. poisoning in the Moroccan population to determine the characteristics and the prognostic factors of this type of poisoning in our context.

Methods

Study population and data collection

The present retrospective study was performed in Morocco. All cases related to acute *A. gummifera* L. poisoning received in the Moroccan poison control centre from January 1981 to December 2009 were included.

The survey collected information on sociodemographic characteristics (age, sex, origin), circumstances (accident, therapeutic use, suicide), clinical symptoms, therapeutic aspects (symptomatic treatment and gastric decontamination) and outcome (mortality rate). The patient's clinical state was classified according to the Poisoning Severity Score [8]. Treatment at the centre is based on symptomatic treatment such as the correction of hypoglycaemia by infusion of glucose solution, the administration of oxygen or intubation-ventilation in case of respiratory or neurological distress and correction of metabolic acidosis and hydroelectrolyte disorders.

Statistical analysis

Epi 2000, version 3.3.2 program was used to perform the analysis. The chi-squared test was used to assess the significance of differences in the distribution of selected sociodemographic characteristics, circumstances, clinical symptoms, therapeutic aspects and frequency of deaths among the participants.

A logistic regression was performed with death versus living as the dependent variable. We compared the groups of survivors and deceased to determine some prognostic factors. The explanatory factors were coma, hepatitis, gastric decontamination, and residence area. Odds ratios (OR) with 95% confidence interval (CI) and degree of significance (*P*-value) was determined for each variable. A *P*-value of < 0.05 was considered significant.

Results

In our study 467 cases of *A. gummifera* L. poisoning were included, representing 10.6% of all cases of plant poisoning collected over the same period. Declarations came from health professionals in 94.4% of cases and from the public in 5.6%.

Profile of poisoning cases

Although the number of cases fluctuated annually, they decreased slightly after 2006 (Figure 1). The number varied between 2 cases in 1982 to 53 cases in 1996 (11.1%). This type of poisoning was found in all regions of our country with a clear predominance in the region of Fez-Boulemane (27.4% of cases), followed by the regions of Taza-Al Hoceima-Taounate (16.3%) and Marrakech-Tensift-Al Haouz (10.2%).

The mean age of cases was 15.3 (SD 12.5) years, ranging from 1 to 70 years. The most vulnerable age group was children (63.4% of cases), followed by adults (22.4%). Children aged 4–10 years and from rural areas were more of

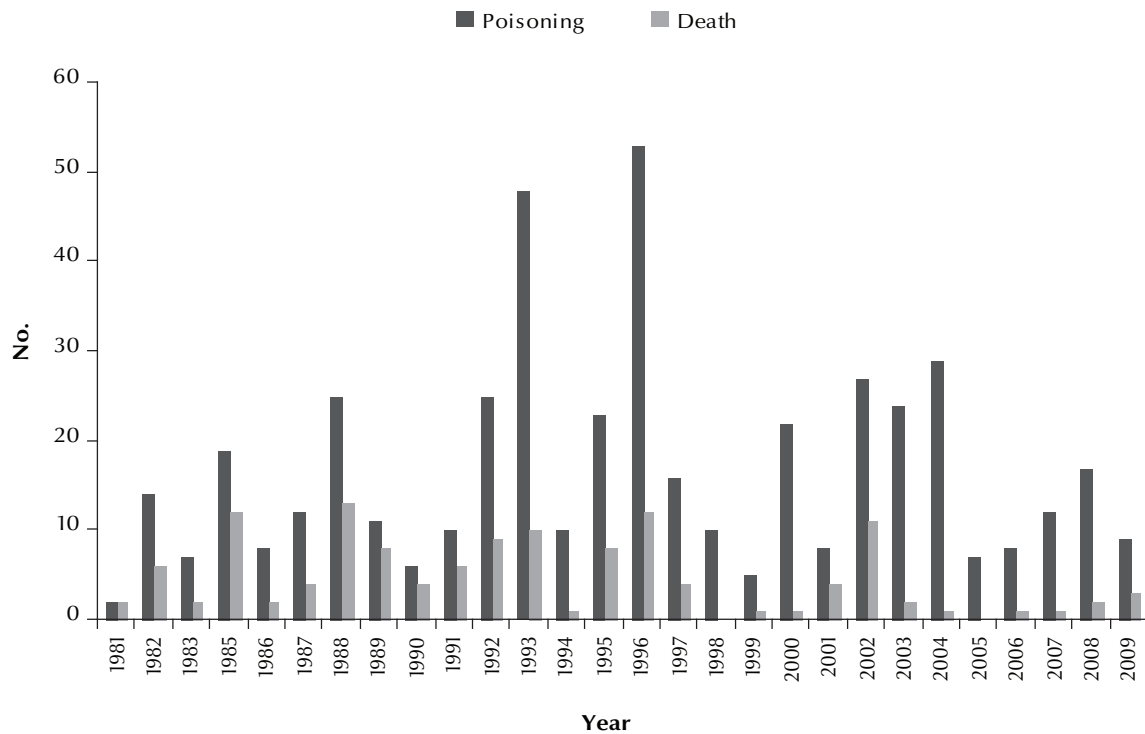


Figure 1 Distribution of cases of poisoning ($n = 467$) and deaths ($n = 130$) due to *Atractylis gummifera* L. by year (1981–2009)

the cases (51.7%). The sex ratio (male/female) was 0.76 in favour of females (203 males versus 264 females).

The route of intoxication was indicated in 380 cases. Ingestion was the most common route of exposure (98.0%), followed by topical administration in only 2.0% of cases. Accidental exposure was the most common circumstance (75.5%); therapeutic use was noted in 18.1% and the plant was used for attempted abortion in 7.4%. Adult females were more implicated in therapeutic use (48.4%) and male children in accidental exposure (51.6%).

Patients were symptomatic in 67.3% of cases; the evaluation of clinical severity at admission according to Poisoning Severity Score showed that 22.3% were in grade 2 with pronounced signs and 21.0% of patients were in grade 3 with life-threatening symptoms. Hepatodigestive disorders were the most commonly observed symptoms (57.0%) followed by neurological disorders (26.9%) (Table 1). Hepatitis was found

in 106 cases, associated with jaundice and elevation of serum transaminases and bilirubin. Prothrombin time was specified in 61.1% of cases and it was less than 50% in 96 cases, while fulminant hepatitis was described at admission in 36 cases. Furthermore, hyperglycaemia followed by hypoglycaemia was reported in 30.3% of cases and renal failure in 16.2% of cases.

The management delay after intoxication was less ≤ 4 hours in 72.2% of the cases. Gastric decontamination was performed in 40.3% of cases and symptomatic treatment was made in 71.1% of cases. The outcome was specified in 332 cases, of which 130 deaths were recorded. The mortality rate was 39.2%. The majority of deaths (81.1%) occurred in children aged < 15 years following accidental exposure. The distribution of cases and deaths according to year is shown in Figure 1. The number of deaths varied in each year with a pronounced decrease after 2002.

Risk factors associated with death

In order to identify clinical risk factors associated with death, we compared the 2 groups (survivors and deaths) using univariate analysis. The parameters statistically associated with death and elucidated by P -value < 0.05 are reported in Table 2. Death was significantly more common in children aged < 15 years ($P < 0.001$), among females ($P = 0.05$), rural residents ($P < 0.001$) and in cases of accidental poisoning ($P < 0.001$). Death was significantly associated with presence of tachycardia, dizziness, miosis, haemorrhage/bleeding, history of hepatitis or coma. Patients with abdominal pain and having gastric decontamination were significantly less likely to die.

Table 3 gives the adjusted OR from multivariate logistic regression models to tease out the adjusted association between different characteristics and survival status. The analysis revealed that a history of coma was

Table 1 Symptoms presented by patients affected by *Atractylis gummifera* L. poisoning

Signs and symptoms	No. with signs ^a	%
Hepato-digestives disorders		
Hepatitis	106	12.6
Vomiting	150	17.9
Nausea	88	10.5
Abdominal pain	73	8.8
Intestinal bleeding	38	4.5
Diarrhoea	24	2.9
Total	479	57.0
Neurological disorders		
Coma	76	9.1
Dizziness	32	3.8
Headache	30	3.6
Mydriasis	26	3.1
Sensorymotor deficit		
Drowsiness	16	1.9
Convulsions	14	1.7
Restlessness	14	1.7
Total	226	26.9
Cardio-respiratory disorders		
Dyspnoea	40	4.8
Collapse: hypotension	34	4.1
Hypertension	10	1.2
Arrhythmia	6	0.7
Bronchospasm	3	0.3
Apnoea	2	0.2
Cyanosis	2	0.2
Total	97	11.5
General signs		
Anuria	11	1.3
Dry mouth	9	1.1
Sialorrhoea	8	1.0
Asthaenia	7	0.8
Skin rash	3	0.4
Total	38	4.5
All signs	840	100.0

^aEach patient may have presented 1 or more clinical signs, and therefore the number of symptoms exceeded the number of patients.

significantly associated with higher risk of death compared with the subjects without a history of coma (OR = 20.5, 95% CI: 5.0–84.0), independent of the potential confounders. Subjects with a history of hepatitis had a greater likelihood of death than

those without (OR = 52.7, 95% CI: 15.0–185). Living in a rural area (OR = 7.26, 95% CI: 2.68–19.6) was also a risk factor for death. Gastric decontamination was the only protector factor of death (OR = 0.26, 95% CI: 0.07–0.96).

Discussion

Despite the plant's well-known toxicity, ingestion of *A. gummifera* L. continues to be a common cause of poisoning in Morocco. In our study 467 cases were compiled, mainly in the Fez-Boulemane and the Taza-Al Hoceima-Taounate regions. This number is likely to be an underestimate because a large number of patients who died were not declared to the poison centre. Poisoning by this plant is common in the Mediterranean region and frequently fatal. It has been described since the mid-19th century [9]. About 200 cases have been reported since then [4,10], mainly by accidental substitutions or due to children chewing the sweet gum obtained from the latex of its subterranean parts. Although most poisoning cases occur in North Africa [10] they have also been reported among European countries: Greece [11]; Spain [12]; Italy [13]. If used internally it is extremely toxic, even at very low doses.

A report by Hamouda et al. stated that from 1983 to 1998 the Tunisian poisoning centre collected 56 medical records of patients admitted to the toxicological intensive care unit for poisoning with 11 species of plants [14]. The principal plants involved were *A. gummifera* (18 cases; 32%), *Datura stramonium* L. (14 cases; 25%) and *Ricinus communis* L. (5 cases; 9%). Of these 56 cases 16 were lethal and all of them involving *A. gummifera*.

Because *A. gummifera* L. is easily confused with a wild artichoke *Scolymus hispanicus* L., most poisonings are unintentional (75.5% in our study) and involved mainly children because this thistle has sweet-tasting juice and children enjoy chewing the chewing-gum-like substance from the roots [15]. Therapeutic circumstances were reported in 18.1% and attempted

Table 2 Univariate analysis of factors associated with mortality among patients affected by *Atractylis gummifera* L. poisoning

Variables	Survived		Died		P-value
	No.	%	No.	%	
Demographic data					
Age group (years)					
Child (0–14)	104	53.6	109	87.9	0.001
Teenager (15–19)	27	13.9	6	4.8	
Adult (20–74)	63	32.5	9	7.3	
Sex					
Female	123	63.1	65	52.4	0.05
Male	72	36.9	59	47.6	
Residence					
Urban	82	72.6	26	35.1	0.001
Rural	31	27.4	48	64.9	
Circumstances					
Voluntary	52	27.7	6	5.1	0.001
Accidental	136	72.3	111	94.9	
Clinical signs					
Abdominal pain					
No	152	76.7	123	94.6	0.001
Yes	46	23.2	7	5.4	
Tachycardia					
No	188	94.9	115	88.5	0.030
Yes	10	5.1	15	11.5	
Dizziness					
No	190	96.0	114	87.7	0.005
Yes	8	4.0	16	12.3	
Miosis					
No	195	98.5	122	94.6	0.045
Yes	3	1.5	7	5.4	
Coma					
No	190	96.0	90	69.2	0.001
Yes	8	4.0	40	30.8	
Haemorrhage/ bleeding					
No	197	99.5	122	93.8	0.002
Yes	1	0.5	8	6.2	
Hepatitis					
No	182	91.9	41	31.5	0.001
Yes	16	8.1	89	68.5	
Gastric decontamination					
No	154	77.8	115	88.5	0.014
Yes	44	22.2	15	11.5	

Data missing in some categories.

abortion in 7.4% of cases. In fact, in folk medicine, *A. gummifera* has been used to treat several conditions including intestinal parasites, ulcers, snake-bite poisoning, hydrops and drowsiness.

In traditional Arabic medicine it was used to cauterize abscesses. The plant was also known for its antipyretic, diuretic, purgative and emetic properties [16]. It is also used against parasites

in folk veterinary medicine [17]. In the popular medicine of North Africa it is still used to treat syphilitic ulcers, induce abortion and bleach the teeth [18].

Table 3 Multivariate logistic regression analysis of factors associated with mortality among patients affected by *Atractylis gummifera* L. poisoning

Variables	ORa	95% CI	P-value
Coma	20.5	5.0–84.4	0.001
Hepatitis	52.7	15.0–185	0.001
Gastric decontamination	0.26	0.07–0.96	0.04
Rural origin (rural versus urban)	7.26	2.68–19.6	0.001

ORa = adjusted odds ratio; CI = confidence interval.

Several case reports of poisoning have been published in the literature and provide useful information on the symptoms and laboratory findings that help to identify victims of *A. gummifera* poisoning. The symptoms begin 6–36 hours after the ingestion of the extract of the *A. gummifera* rhizome [16]. The signs and symptoms found in our series corroborate those found in the literature already cited. The laboratory findings (marked increased in serum glutamic oxaloacetic transaminase, serum glutamic-pyruvic transaminase and bilirubin) may indicate severe hepatocellular damage and acute renal failure [19]. In our series, hepatitis was present in 106 patients and was associated with a high mortality.

A. gummifera L. poisoning is responsible for a heavy burden of morbidity and mortality and to our knowledge the factors that determined death were never investigated. Our study is the first one to focus on the study of prognostic factors. The multivariate analysis

revealed that mortality in *A. gummifera* L. poisoning correlated with coma and hepatitis and the presence of these signs increased the risk of death. This can be explained by the pathologic action of atractyloside and carboxyatractyloside, which involves inhibition of adenosine diphosphate triphosphate conversion through inhibition of P450 cytochrome, thus leading to damage of tissues. The organs with the greatest oxygen requirements appear to be especially sensitive to damage; these include the brain and liver.

No specific pharmacological treatment (antidote) is currently available to treat *A. gummifera* intoxication and all therapeutic approaches including fluid and electrolyte replacement, cardiovascular and respiratory support, seizure control and conventional therapeutic methods for severe hepatic and renal failure are only symptomatic [20]. Some authors recommend that standard therapeutic practice should include induction of vomiting, bowel

evacuation, gastric decontamination and administration of activated charcoal [21]. The majority of these treatments were performed in our patients, except activated charcoal, because it is not available in our country. In our study, gastric decontamination was a protector factor against death, and indeed, by reducing the toxic load, this type of treatment improves the prognosis of poisoned patients. Symptomatic treatment is still insufficient in patients who have taken quantities theoretically lethal of the poison. In spite of the progress achieved in the fields of toxicology and associated therapy, *A. gummifera* L. poisoning is still responsible for a high rate of mortality (39.2% in our study). New therapeutic approaches could come from immunotherapy research: some studies have already tried to produce polyclonal Fab antibody fragments against the toxic components of *A. gummifera* [22].

Competing interests: None declared.

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Case report

Case of acquired lobar emphysema mimicking pneumothorax in a neonate

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Introduction

Despite the improvements in prevention of acute respiratory disease in preterm infants, the incidence of bronchopulmonary dysplasia (BPD) remains largely unchanged. Acquired lobar emphysema (ALE) is an increasingly recognized complication of advanced BPD. Barotrauma, oxygen toxicity and lung immaturity are presumed to play an important role in the development of ALE in children with BPD and most cases present overinflation [1,2]. We report on an infant with BPD who developed ALE mimicking pneumothorax.

Case report

Following a 30-week of gestation complicated with premature rupture of membranes for 3 days, a 1275 g male infant was delivered by caesarean section. The initial chest radiography showed a grade IV respiratory distress syndrome (RDS), requiring a total of 3 surfactant administrations. The initial situation was complicated by a systemic inflammatory response syndrome. Ventilatory support was performed to treat respiratory acidosis and severe RDS. On postnatal day 28, when the child had been on mechanical ventilation, a right pneumothorax developed. A chest tube was inserted and removed after 3 days. On postnatal day 31, radiographic evidence of BPD was noted (Figure 1).

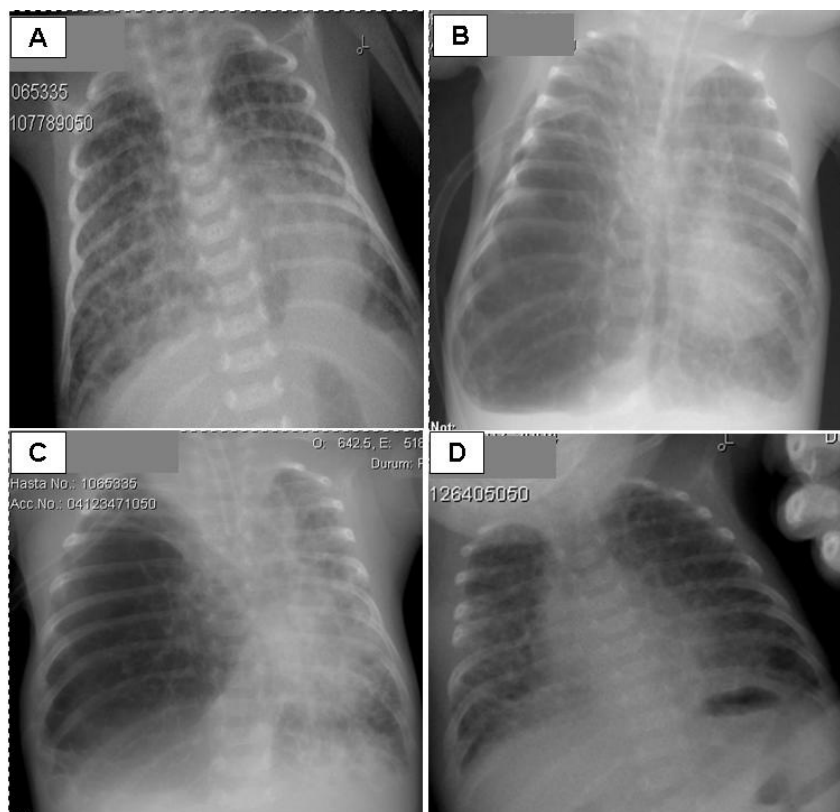


Figure 1 Chest radiographs of a case of acquired lobar emphysema mimicking pneumothorax in a neonate. (A) Postnatal day 21: bilateral diffuse cystic changes in lung parenchyma, consolidation on the left retrocardiac side. (B) Postnatal day 56: emphysema on the right lower lobe of the lung, deviation of the heart and mediastinal structures to the left, diffuse cystic parenchymal changes on the right upper lobe and left lobe of the lung. (C) Postnatal day 59: emphysema on the right lower lobe of the lung, deviation of the heart and mediastinal structures to the left. (D) Postnatal day 63: bilateral diffuse cystic parenchymal changes and no emphysema after right lower lobectomy

Although vitamin A supplementation and dexamethasone treatment were administered, the infant did not tolerate extubation. On postnatal day 56, severe acute hypoxaemia developed. Chest radiography confirmed the diagnosis of right pneumothorax and a chest tube

was inserted. However, the infant deteriorated clinically and repeated radiography revealed lobar emphysema on the right lower lung. The infant underwent right lower lobectomy and a marked clinical improvement after surgery was evident. The patient was extubated on

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postnatal day 67. The infant was then transferred to another hospital due to his family's request on postnatal day 125. On discharge, he was clinically stable and receiving only supplemental oxygen.

Discussion

The pulmonary air leak syndromes, including pneumomediastinum, pneumothorax, pulmonary interstitial emphysema and pneumopericardium, comprise a spectrum of disease with the same underlying pathophysiology. They are common in preterm neonates with RDS during treatment with mechanical ventilation. The incidence is about 10% of the ventilated preterm infants treated with surfactants [3]. This is a case report of a preterm newborn

developing RDS complicated with BPD and ALE during mechanical ventilation. Initially he was managed with chest tube drainage due to diagnosis of pneumothorax. However repeated radiologic examination revealed the diagnosis of ALE.

ALE is an usual complication of mechanical ventilatory support in neonates with RDS. Although numerous therapeutic approaches to this complication have been described, there is no widely accepted management strategy in current practice. Therapeutic options include positioning of the neonate on the affected hemithorax, selective ventilation of the unaffected lung with conventional ventilation, selective occlusion of the affected mainstem bronchus, surgical resection of the affected lung portion, application of high-frequency ventilation to the trachea and administration of

dexamethasone [4–9]. In our case, resection of the large emphysematous bulla was successfully performed without any perioperative complications. Considering the surgical approach the postoperative outcome was encouraging, although the long-term outcome of the child remains unclear. In the literature concerning infants who failed medical management, lobectomy is clearly beneficial [1].

In conclusion, ALE should be kept in mind as a complication in infants with severe BPD on mechanical ventilation. Early diagnosis of the ALE is important for conservative management. A misdiagnosis of pneumothorax should be avoided. Obviously, prevention is better than treatment. Therefore clinical trials in patients with BPD will provide additional therapeutic options for the treatment and prevention of complications of BPD.

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