Antimicrobial resistance pattern of Gram-negative bacteria of nosocomial origin at a teaching hospital in the Islamic Republic of Iran

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أنماط الاستعصاء على مضادات المكروبات في جراثيم سلبية الغرام مستشفويّة المنشأ من أحد المستشفيات التعليمية في جمهورية إيران الإسلامية

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الخلاصة: إن بزوغ الاستعصاء على مضادات المكروبات يمثِّل إحدى المشكلات العالمية في المجتمع وفي المستشفيات؛ وتقيِّم هذه الدراسة الاستعصاء على المضادات الحيوية في جراثيم سلبية الغرام المكتسبة على مدى ستة أشهر في مستشفى شريعتي التعليمي في طهران، في جمهورية إيران الإسلامية. حيث أجرى الباحثون اختبارات الحساسية لمضادات المكروبات لدى 570 من الجراثيم السلبية الغرام المستفردة من عينات سريرية من مرضى أصيبوا بالعدوى إثر مكثهم 72 ساعة في المستشفى. واتضح أن الإيشريكيات القولونية تمثِّل أكثر المكروبات السلبية الغرام المستفردة (42.6٪)، كما شوهد أعلى معدل للاستعصاء بين مستفردات الجراثيم السلبية الغرام في وحدات الرعاية المركَّزة في أنواع الراكدة Acinetobacter باعتبارها أكثر الجراثيم استعصاءً. وكان الجنتاميسين أكثر المضادات الحيوية فعالية ضد الإيشريكيات القولونية وجميع المستفردات الأخرى، في حين كان السيبروفلوكساسين فعّالاً ضد طائفة واسعة من الأنواع الأخرى. ويتبيَّن مما تقدَّم أن العدوى المستشفويّة بالجراثيم السلبية الغرام المستعصية على المضادات الحيوية منتشرة في هذا المستشفى التعليمي في طهران.

ABSTRACT The emergence of antimicrobial resistance is a global problem in the community and in hospitals. Antibiotic resistance of Gram-negative bacteria from nosocomial infections were evaluated during a 6-month period at Shariati teaching hospital, Tehran, Islamic Republic of Iran. Susceptibility tests were performed on 570 Gram-negative isolates obtained from clinical samples of patients infected after at least 72 hours stay in the hospital. *Escherichia coli* was the most frequently isolated Gram-negative organism (42.6%). The highest rate of resistance in Gram-negative isolates was seen in the intensive care unit, with *Acinetobacter* spp. as the most resistant organisms. Gentamicin was the most effective antibiotic against *E. coli* and all other isolates, while ciprofloxacin was also effective against a wide range of other species. Antibiotic resistant Gram-negative nosocomial infection is prevalent in this teaching hospital in Tehran.

Schéma de résistance aux antimicrobiens des bactéries à Gram négatif d'origine nosocomiale dans un centre hospitalier universitaire de la République islamique d'Iran

RÉSUMÉ La résistance aux antimicrobiens est un problème mondial pour la population comme pour les hôpitaux. La résistance aux antibiotiques des bactéries à Gram négatif responsables d'infections nosocomiales a été évaluée pendant six mois à l'hôpital universitaire Shariati, à Téhéran (République islamique d'Iran). Des épreuves de sensibilité ont été réalisées sur 570 isolats à Gram négatif obtenus à partir d'échantillons cliniques prélevés chez des patients infectés ayant passé au moins 72 heures à l'hôpital. La bactérie à Gram négatif la plus fréquemment isolée était *Escherichia coli* (42,6 % des échantillons). Le taux de résistance le plus élevé a été observé dans l'unité de soins intensifs, *Acinetobacter spp*. étant l'organisme le plus résistant. La gentamicine était l'antibiotique le plus efficace contre *E. coli* et contre tous les autres isolats, mais la ciprofloxacine était également efficace contre un large spectre d'autres espèces. Les infections nosocomiales dues à des bactéries à Gram négatif résistantes aux antibiotiques sont fréquentes dans cet hôpital universitaire de Téhéran.

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Introduction

Nosocomial or health care-associated infections account for a high morbidity and mortality rate among hospitalized patients [1]. The response to therapy of these infections has changed and antibiotic resistance incidence has increased at an alarming pace over the past 10 years. About 50%–60% of hospital-acquired infections are caused by resistant strains [2]. A number of factors have been reported that were related to infection with resistant microorganisms, including previous use of antibiotics [3–6], corticosteroid therapy [7], mechanical ventilation [5–7], length of hospital stay [8] and use of invasive devices such as catheters [9,10].

Antimicrobial resistance results in increased morbidity, mortality and costs of treatment. Preventing the emergence and dissemination of resistant organisms is critical for control of hospital infections. Appropriate antimicrobial stewardship that includes optimal selection, dose and duration of treatment, as well as control of antibiotic use, will prevent or slow the emergence of resistance among microorganisms [2].

The prevalence of antimicrobial resistance varies across different settings and studies. Having an awareness of antimicrobial resistance patterns, particularly in hospitals, is crucial for the selection of appropriate antibiotic therapy to improve treatment outcomes, reduce morbidity and mortality, shorten the hospitalization period and consequently reduce the cost of care. The aim of this study was to determine the antimicrobial resistance pattern of Gram-negative bacteria at a teaching hospital in Tehran, Islamic Republic of Iran. Surveillance programmes for regular evaluation of antibiotic resistance patterns are lacking in the Islamic Republic of Iran and this is one of the few comprehensive studies that have been conducted in this area.

Methods

Setting

This prospective case-series study was done at Shariati general teaching hospital affiliated to Tehran University of Medical Sciences during December 2007 to May 2008. This is a 500-bed hospital with different medical wards including intensive care unit (ICU), gastroenterology, haematology/oncology and bone marrow transplantation, rheumatology, cardiology, nephrology, neurosurgery, cardiac surgery, general surgery and gynaecology and obstetrics.

Sample

Biological samples were collected from patients hospitalized in different wards of the hospital who were diagnosed with nosocomial infection. All aerobic Gram-negative isolates collected from patients with at least 72 hours stay in the hospital and without evidence of infection at the time of admission were included in the study. Only 1 isolate per patient per site was included.

For each patient a samples was collected from the most likely site of infection: blood, urine, cerebrospinal fluid (CSF), respiratory tract secretions (collected by bronchoscopy or endotracheal suction), tips of central venous catheters, wound secretions, intra-abdominal abscesses, articular fluid or other clinically relevant sites.

To determine the most important contributing factors to antibiotic drug resistance, demographic data and medical history (including history of invasive procedures and any comorbidity) were recorded for patients with isolates found to be resistant.

Laboratory methods

Bacterial species were identified by different microbiologic tests, including growth in eosin–methylene–blue and MacConkey media, Gram stain, urease production, H_2S production in sulphur–indole motility media, indole production, motility, methyl red test, citrate utilization and decarboxylase production.

Mueller–Hinton agar was used as the growth medium and the antimicrobial susceptibility testing was performed on positive cultures which yielded Gram-negative organisms, using the Kirby–Bauer (disk diffusion) method, in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. Susceptibility testing was done for amikacin, gentamicin, tobramycin, ceftazidime, ceftriaxone, cefazolin, cefuroxime, piperacillin, piperacillin/tazobactam, ciprofloxacin, nalidixic acid, and nitrofurantoin.

The results were interpreted after 18–24 hours of incubation at 35 °C. The zone diameter measured around each disk was measured and classified using CLSI guidelines as susceptible, intermediate or resistant according to the zone size diameters [11,12].

Analysis

We used *SPSS*, version 11.5 for data analysis. The results were reported as frequencies. Multivariate analysis was performed to determine variables with independent significant correlations. *P* values ≤ 0.05 were considered as statistically significant.

Results

Organisms isolated

A total of 570 samples were obtained. Gram-negative organisms were isolated mainly from the urinary tract (45.6%), blood (21.5%), and respiratory tract (19.3%). The remaining isolates (13.1%) were obtained from wound (6.3%), CSF (4.8%) and articular fluid (2.5%) or other sources (2.5%).

Table 1 shows the organisms isolated from 556 of the samples for which the organism could be identified. *Escherichia coli* was the most frequently isolated Gram-negative organism (42.8%), followed by *Acinetobacter* spp. (17.1%), *Klebsiella* spp. (16.5%), *Pseudomonas*

aeruginosa (14.2%) and *Enterobacter* spp. (7.6%). The remaining 1.8% of isolates included *Proteus vulgaris* and *Morganella morganii*.

In terms of the infection site *E. coli* was the most frequent infecting Gram-negative organism isolated from urine samples and wound secretions, while *E. coli*, *Acinetobacter* spp., *Klebsiella* spp., *P. aeruginosa* and *Enterobacter* spp., all were isolated from blood specimens in similar proportions (Table 1). *Acinetobacter* spp. were the most frequently isolated Gram-negative organisms from respiratory tract secretions.

Antibiotic susceptibility

The overall susceptibility of the isolated Gram-negative organisms to antimicrobial agents was 51.3% for gentamicin, 47.5% for ciprofloxacin, 32.0% for amikacin, 29.7% for ceftazidime, 22.1% for ceftriaxone, 13.8% for nitrofurantoin, , 9.0% for nalidixic acid, 8.1% for cefazolin, 7.7% for tobramycin, 4.3% for piperacillin/tazobactam, 2.5% for piperacillin and 1.3% for cefuroxime (Table 2). Gentamicin was the most effective antibiotic against *E. coli*, while ciprofloxacin was also effective against a wide range of other Gram-negative bacteria isolated. The least effective agent for all isolated Gram-negative microorganisms was cefuroxime.

Patient evaluation

A total of 60 patients with resistant isolates were evaluated. Among the studied patients, 58.3% (35 cases) were male. The mean age of the patients was 49.5 (SD 22.3) years.

Of the patients 35.0% had history of recent hospitalization (for at least 48 hours, within the last 3 months) and 40.0% of them had a history of a recent surgery (within the previous 3 months). Concerning the presence of comorbidity, chronic heart failure (28.3%), diabetes (21.7%), chronic renal failure (21.7%) and impaired immune system function due to long-term corticosteroid use (18.3%) were the most common comorbid conditions reported. Additionally, 16.7% of patients were receiving total parenteral nutrition during hospitalization.

Of these potential predisposing factors, history of recent hospitalization (OR = 3.9, 95% CI: 1.8–14.5), history of recent surgery (OR = 2.3, 95% CI: 1.1–8.6) and long-term corticosteroid therapy (OR = 1.9, 95% CI: 1.2–6.9) were significantly associated with presence of bacterial resistant isolates (Table 3).

Discussion

Nosocomial infections are a serious threat to hospitalized patients. The emerging problem of antimicrobial resistance further complicates the issue because most deaths associated with nosocomial infection are caused by antibiotic-resistant microorganisms [1,2].

In our study, based on the culture sources, the most frequent nosocomial Gram-negative infections were urinary tract infection (45.6%), followed by bacteraemia (21.5%) and pneumonia (19.3%). In a recent study in ICU patients of 2 other teaching

Table 1 Frequency of Gram-negative microorganisms isolated from biological samples at different infection sites in patients with nosocomial infection (n = 556)

Infection site	Tested[a]	Escherichia coli		Acinetobacter spp.		Klebsiella spp.		Pseudomonas aeruginosa		Enterobacter spp.		Others	
	No.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Urine	254	160	63.0	18	7.1	42	16.5	20	7.9	9	3.5	5	2.0
Blood	121	24	19.8	24	19.8	21	17.4	24	19.8	26	21.5	2	1.7
Respiratory secretions	93	18	19.4	35	37.6	15	16.1	19	20.4	6	6.5	0	0.0
Wound secretions	32	15	46.9	5	15.6	5	15.6	4	12.5	0	0.0	3	9.4
Throat	12	2	16.7	2	16.7	4	33.3	4	33.3	0	0.0	0	0.0
Cerebral & articular fluids	8	3	37.5	1	12.5	1	12.5	2	25.0	1	12.5	0	0.0
Other	36	16	44.4	10	27.8	4	11.1	6	16.7	0	0.0	0	0.0
Total	556	238	42.8	95	17.1	92	16.5	79	14.2	42	7.6	10	1.8

aNumber of samples tested.

Table 2 Antibiotic susceptibility rates of nosocomial bacterial isolates (n = 556)

Antibiotic	Escherichia coli (n = 238)		Acinetobacter spp. (n = 95)		Klebsiella spp. (n = 92)		Pseudomonas aeruginosa (n = 79)		Enterobacter spp. (n = 42)		Others (n = 10)		Total (n = 556)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Gentamicin	125	52.5	14	14.7	48	52.2	34	43.0	26	61.9	8	80.0	285	51.3
Ciprofloxacin	90	37.8	27	28.4	48	52.2	43	54.4	31	73.8	7	70.0	264	47.5
Amikacin	81	34.0	15	15.8	29	31.5	24	30.4	25	59.5	4	40.0	178	32.0
Ceftazidime	75	31.5	5	5.3	32	34.8	24	30.4	22	52.4	7	70.0	165	29.7
Ceftriaxone	64	26.9	2	2.1	30	32.6	6	7.6	15	35.7	5	50.0	123	22.1
Nitrofurantoin	70	29.4	2	2.1	4	4.3	0	0.0	1	2.4	0	0.0	77	13.8
Nalidixic acid	34	14.3	0	0.0	14	15.2	1	1.3	0	0.0	1	10.0	50	9.0
Cefazolin	21	8.8	2	2.1	13	14.1	5	6.3	1	2.4	3	30.0	45	8.1
Tobramycin	8	3.4	6	6.3	4	4.3	12	15.2	12	28.6	1	10.0	43	7.7
Pipracillin/tazobactam	5	2.1	4	4.2	0	0.0	9	11.4	6	14.3	0	0.0	24	4.3
Piperacillin	1	0.4	2	2.1	0	0.0	6	7.6	5	11.9	0	0.0	14	2.5
Cefuroxime	2	0.8	0	0.0	2	2.2	2	2.5	0	0.0	1	10.0	7	1.3

hospitals of Tehran, Islamic Republic of Iran (Sina and Imam Khomeini hospitals) performed by Hadadi et al., septicaemia (33.0%) and pneumonia (29.5%) were the most frequent nosocomial infections [13]. In the study of Vesal et al. performed on Gram-negative isolates of ICU patients at Shariati hospital during a 6-month period from October 2003 to April 2004, pneumonia was the most frequent nosocomial infection [14]. These slightly different results could be explained by the difference in the patient population of these studies; our study included patients from all medical wards whereas other studies were of ICU patients only.

In our study, *E. coli* was the most frequent isolate in urinary tract infection and a major isolate in bacteraemia. This shows that urosepsis is a major cause of nosocomial infections.

Acinetobacter spp. and *P. aeruginosa* were the most frequently isolated Gram-negative bacteria from the respiratory tract in the current study. This is in agreement with the study of Vesal et al. that reported *Acinetobacter* as the most frequent isolated species [14]. In contrast, in the study of Hadadi et al. *Klebsiella* spp. and *P. aeruginosa* were the most frequent isolates from respiratory tract specimens [13]. Therefore, it can be concluded that *Acinetobacter* spp. and *P. aeruginosa* are major pathogens responsible for nosocomial pneumonia.

In the current study, *Acinetobacter* spp. were the most antibiotic-resistant Gram-negative microorganisms. This is in agreement with a study in Chicago in 2004 that reported a high resistance rate of *Acinetobacter* spp. to all commonly used antibiotics with a resultant high morbidity and mortality rate [15]. Similar results have been reported in a Spanish study in 2004 [16].

In our study, *Acinetobacter* spp. was both the most frequently isolated agent from the respiratory tract and the most resistant of all isolates. This highlights the need for new effective antimicrobial agents and strategies to prevent and treat common respiratory tract infections such as hospital-acquired pneumonia.

Some other studies have reported *P. aeruginosa* as the most resistant organism [17,18]. This is consistent with our study that showed this pathogen as the second most resistant organism. In the current study, the most effective antibiotic against this pathogen was ciprofloxacin with a susceptibility of 54.4%, while in the study of Hadadi et al. the susceptibility of *P. aeruginosa* to this antibiotic was 33.3% [13].

Although ciprofloxacin, amikacin and gentamicin were the most effective agents against Gram-negative isolates in our research, nevertheless between 49%–68% of all isolates were resistant to these antibiotics.

Table 3 Variables associated with presence of antibiotic resistance strains in patients with nosocomial infection (*n* = 60)

Variable	Resistant		Not resistant		Crude OR (95% CI)	*P*-value
	No.	%	No.	%		
Age group (years)						
< 50	24	40.0	210	42.3	1.1 (0.8–1.6)	0.33
50–59	20	33.3	160	32.3	0.8 (0.3–1.2)	0.51
60–69	12	16.7	100	20.2	1.3 (0.5–1.5)	0.30
70+	4	6.6	26	5.3	0.9 (0.4–1.5)	0.44
Sex						
Male	35	58.3	256	51.6	0.4 (0.1–1.4)	0.31
Female	25	41.7	240	48.8	1.3 (0.4–1.9)	0.81
Clinical history						
History of recent hospitalization	21	35.0	63	13.8	3.9 (1.8–14.5)	0.003
History of recent surgery	24	40.0	73	16.0	2.3 (1.1–8.6)	0.01
Long-term corticosteroid therapy	11	18.3	57	12.5	1.9 (1.2–6.9)	0.004

OR = odds ratio; CI = confidence interval.

As expected, cefuroxime was the least effective agent against isolates and its empirical use in nosocomial Gram-negative infections should therefore be avoided.

Our study detected several factors, including history of surgery and hospitalization, and some comorbidities, including heart failure, diabetes, chronic renal failure and long-term corticosteroid use, as possible contributing risk factors to antimicrobial resistance in patients from whom resistant organisms were isolated. However, a larger study with a larger sample is needed to confirm these data.

In conclusion, the rate of antimicrobial resistance is high among nosocomial Gram-negative bacteria in this hospital, complicating the decision to start appropriate antibiotics to manage nosocomial infections empirically. A surveillance system is recommended to record and report the antimicrobial resistance pattern of hospital isolates periodically, as this pattern changes continually over time.

Acknowledgements

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Communicable diseases in the Eastern Mediterranean Region. Prevention and control 2005–2009

In the World Health Organization (WHO) Eastern Mediterranean Region, communicable diseases are among the major causes of mortality and morbidity and pose major impediments to social and economic wellbeing. *Communicable diseases in the Eastern Mediterranean Region. Prevention and control 2005–2009* provides an overview of the situation of communicable diseases, the progress made by countries with support from WHO and partners, and the key challenges for disease prevention and control during the period 2005–2009. The report describes the status in relation to 6 visions for communicable diseases prevention and control in the Region: Elimination and eradication of specific diseases; Expanding disease-free areas; Providing a safe vaccine for every childhood disease for every child; Curbing the HIV/AIDS epidemic; Halving the burden of tuberculosis – working towards elimination and; Containing new and re-emerging disease threats.

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