ORIGINAL ARTICLE

Frequency and Antimicrobial Susceptibility Pattern of *Acinetobacter* Species Isolated from Pus and Pus Swab Specimens

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ABSTRACT

Objective: To evaluate the frequency and antimicrobial susceptibility pattern of *Acinetobacter* species isolated from pus and pus swab specimens at a tertiary care setting.

Study Design: Cross-sectional observational study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from July 2008 to July 2012.

Methodology: Data regarding positive culture and antimicrobial sensitivity pattern was retrieved from the pus and pus swab culture records of the Microbiology Department, AFIP, Rawalpindi. Only those pus and pus swab specimens which yielded the growth of *Acinetobacter* species were included in the study.

Results: Out of 2781, 1848 were of pure pus while 933 were pus swab specimens. Out of 2538 culture positive isolates, 276 (10.9%) were identified as *Acinetobacter* species. Among 276 *Acinetobacter* spp., 245 (88.8%) were *Acinetobacter baumannii* and 31 (11.2%) were *Acinetobacter johnsonii*. Male/female ratio of the affected patients was 5.6:1. Doxycycline was the most sensitive antibiotic to which 45% of the tested isolates were sensitive. Sensitivity to all other antimicrobials was 15% or less.

Conclusion: About 11% of soft tissue and wound infections are caused by *Acinetobacter* species in our set up particularly in male. Doxycycline was the most sensitive antibiotic. Sensitivity to all other antimicrobials was 15% or less. *In vitro* sensitivity to carbapenems is very low.

Key Words: *Acinetobacter spp. Nosocomial infection. Pus specimens. Wound infection. Soft tissue.*

INTRODUCTION

The genus *Acinetobacter* are non-fermentative and non-motile, gram-negative coccobacilli, which comprises 27 known and several unnamed provisional species. Clinically, *Acinetobacter baumannii (Ab)* is most often identified as the cause of infection, but other clinically significant species include *A. johnsonii, A. iwoffii, A. radioresistens, A. calcoaceticus, A. haemolyticus, A. lwoffii* and *A. junii*.[1] *A. baumannii* is an opportunistic pathogen of emerging importance in the clinical settings and responsible for upto 20% of infections in intensive care units around the globe.[2] The majority of reported clinical cases involved ventilator associated pneumonia/ pulmonary infections, bloodstream infections, skin and soft tissue infections including burn and surgical wound infections, endocarditis, meningitis and urinary tract infections. Furthermore, infections caused by *Acinetobacter* are not limited to the hospital settings and reports have emerged unfolding cases involving otherwise healthy individuals of all age groups, occurring in community settings, following natural disasters and during wars.[1-3]

Multidrug Resistant *Acinetobacter baumannii* (MDR-Ab) is one of the most important healthcare-associated pathogens and are increasingly reported around the globe. Due to its remarkable abilities to colonize patients as well as healthcare associated environment, cross-transmission and prolonged environmental survival, it causes healthcare associated outbreaks.[4,5] Treatment of infections due to this pathogen is becoming a serious clinical concern, since *A. baumannii* is showing extensive resistance to many of the currently used antibiotics including cephalosporins, aminoglycosides, quinolones and carbapenems. *A. baumannii* is of particular concern due to its predilection to acquire antibiotic resistance determinants.[2] *A. baumannii* has the capacity to develop antimicrobial resistance by various mechanisms, which is mostly related to mobile genetic elements, such as insertion sequences, plasmids and antibiotic resistant islands.[6]

Nosocomial MDR-Ab infection most commonly occurs in intensive care units (ICUs). Outbreaks in ICUs due to MDR-Ab have been reported to be associated with

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various types of indwelling medical devices and medical procedures used in patient management especially for respiratory system.[2-3] Moreover, the resistance of *A. baumannii* to common disinfectants and ability to survive for long periods on dry surfaces make it difficult to eradicate from the hospital environment. Current multidrug resistance of this organism ranges from 48-85% of clinical isolates, with the greatest burden in Asia, Eastern Europe and Latin America. Pan resistance of this organism is on increase, signifying the fact that soon clinicians will face more such infections for which no effective antimicrobial therapeutic option shall be available. So it is imperative to build-up new anti-microbial approaches to fight this emerging threat.[2,8,9] Rational planning for healthcare policy along with interventional measures are mandatory to tackle such infection in resource-poor countries like ours. Imple-mentation of strict infection control practices, judicious use of antibiotics as well as clinical guidance regarding the potential risks for therapeutic failure is very much required in our healthcare settings.[9]

The changing epidemiology and susceptibility patterns of micro-organisms emphasize the necessity of constant microbiological surveillance in all healthcare settings. Prior understanding of susceptibility patterns of infecting organisms is essential for selection of efficient empirical antimicrobial therapy. The rationale of study was to suggest antimicrobials for empirical treatment based upon the antibiogram of the pathogen responsible to cause soft tissue/wound infections.

The objective of the study was to evaluate the frequency and antimicrobial susceptibility pattern of *Acinetobacter* species isolated from pus and pus swab specimens at a tertiary care setting.

METHODOLOGY

This study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan. Sampling technique was non-probability consecutive sampling. All clinical specimens from patients who were admitted in different surgical units and trauma centres were included. All the *Acinetobacter* species isolated from pus and pus swab specimens submitted for culture were included in the study. No discrimination was made on age and gender basis. Contaminated specimens and duplicate speci-mens from same patients were excluded from the study. A total of 276 *Acinetobacter* spp. isolated from pus and pus swab specimens were included in this study. Details like hospital identity number, age, gender of the patients, type and place of submission of specimen were recorded.

All the specimens were inoculated on blood, MacConkey and anaerobic agars as soon as these were received in the department of microbiology and incubated at 35 to 37°C for 24 to 48 hours. Direct microscopy from the Gram stained smears were performed and any evidence of Gram negative pathogen in the smear was recorded. Culture plates were evaluated for evidence of growth after 24 and 48 hours of incubation. Any non-fermenting growth on MacConkey agar was provisionally identified by colony morphology noted on culture plates then by performing microscopy of Gram-stained smear of the colony. Further identification of *Acinetobacter* spp. was made with the help of rapid tests like catalase, oxidase and biochemical reactions on Analytical Profile Index (API) 20 NE.

Antimicrobial susceptibility of the isolate was carried out on Mueller-Hinton (MH) agar (Oxoid, UK) by modified Kirby Bauer disc diffusion technique by inoculating with the test organism (0.5 McFarland standards) to get a semi-confluent growth as per recommendations of Clinical and Laboratory Standards Institute (CLSI).[10] Appropriate antibiotic discs were applied on this MH agar. Following overnight incubation at 35°C ± 2, zone diameters were measured and interpreted as per CLSI guidelines.[10] The data was entered in SPSS (version 17) software and results were interpreted as frequencies and percentages.

RESULTS

A total of 2781 pus and pus swab specimens were submitted to microbiology department for culture and drug susceptibility testing during the period of study from different wards of CMH, Rawalpindi. Out of these 2781 samples, 1848 were pus specimens and 933 were pus swab specimens. Amongst these, 2538 (91%) speci-mens showed growth of different microorganisms. Out of these culture positive isolates, 276 (10.9%) were identified as *Acinetobacter* species. Amongst *Acinetobacter* spp. 245 (88.8%) were of *Acinetobacter baumannii* and 31 (11.2%) were *Acinetobacter johnsonii* (Figure 1).

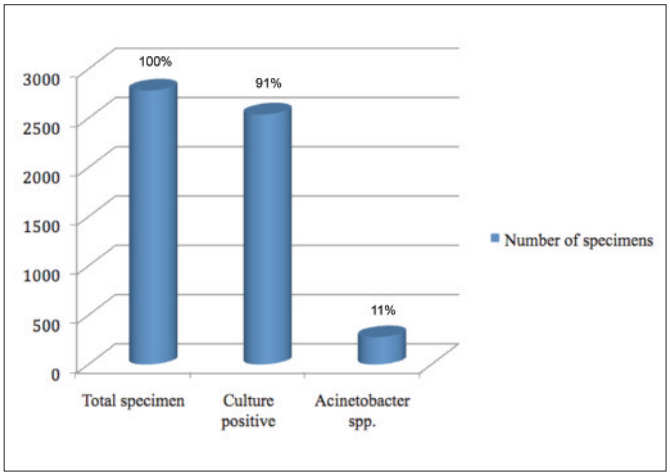

Figure 1: Frequency of *Acinetobacter* spp. in pus and pus swab culture specimens.

Among the clinical specimens yielding growth of *Acinetobacter* spp. 234 (84.8%) specimens were from male patients while 42 (15.2%) were from female patients. Male/female ratio was 5.6:1. Antibiotic susceptibility testing of nine different antibiotics against *Acinetobacter* spp. revealed that Doxycycline was the most sensitive antibiotic to which 45% of the tested isolates were sensitive. Sensitivity to all other antimicrobials was 15% or less. Sensitivity of different drugs is shown in Table I.

Table I: Antimicrobial susceptibility profile of *Acinetobacter* spp. (n=276).

Antibiotic	No. of sensitive isolate	Percentage
Ceftriaxone	9	3.3
Trimethoprim-sulphamethoxazole	32	11.6
Imipenem	28	10.1
Meropenem	28	10.1
Doxycycline	126	45.7
Amikacin	43	15.6
Gentamicin	25	9.0
Ciprofloxacin	17	6.2
Tazobactum-Piperacilin	39	14.1

DISCUSSION

Multidrug resistant Gram-negative pathogens are associated with high morbidity and mortality. Multidrug-resistant *Acinetobacter* spp. has been reported worldwide and is now emerged as one of the hardiest healthcare-associated infections to control and treat. Patients admitted in burn unit, intensive care unit (ICU) and those with central intravenous catheters and respiratory devices are the main targets of this organism.[3,4] Several outbreaks in ICUs, burns units and NICUs have been reported.[3,4,11] The authors also noticed an outbreak during the specified study period.[7] Delay in receiving adequate empirical antimicrobial therapy has an adverse effect on clinical outcomes in hospital-acquired infections caused by *A. baumannii*.[12] In the last few decades, there has been a general trend of increasing incidence of infection due to this pathogen around the globe.[13] Carbapenems were previously known to be effective against MDR-Ab but since the emergence of pan-resistant *Acinetobacter* spp., it is even more difficult to treat this pathogen.[14,15] The Centers for Disease Control and Prevention (CDC) has reported an increasing rate of carbapenems resistant among *A. baumannii* from 9% in 1995 to 40% in 2004.[4] However, the drug resistance is quite variable in different parts of the world.

The majority of *Acinetobacter* spp. isolated from our patients showed resistance to more than one groups of antibiotics. A study from India revealed 87% isolates were MDR and 20% carbapenem resistant.[16] Another study from India showed 33% carbapenem resistance,[17] a study from Korea reported 55.8% carbapenem resistance[12] while carbapenem resistance in this study is about 90% and similar carbapenem resistance among isolates of *Acinetobacter* spp. was reported from the Aga Khan University Hospital at Karachi.[18] A study from Norway revealed that about 9% isolates were *A. baumannii* and 95.6% of these isolates of *A. baumannii* were resistant to ciprofloxacin, nalidixic acid, trimethoprim/sulfamethoxazole and gentamicin, and intermediately susceptible to amikacin;[19] almost similar results were depicted in the present study. A study from Mayo Hospital, Lahore, also reported about 11.8% isolates of *A. baumannii*.[20] Study from Saudia revealed that *A. baumannii* isolates showed high resistance to piperacillin (93.1%), aztreonam (80.5%), ticarcillin, ampicillin, and tetracycline (76.4%, each) and cefotaxime (75%). Only amikacin showed low rate of resistance compared to other antibiotics (40.3%),[21] while in the present results amikacin was resistant in 84.4% of isolates. Another study from India showed that 87% *Acinetobacter* spp. were resistant to third-generation cephalosporins, aminoglycosides and quinolones, indicating high prevalence of MDR.[16] Alarming situation reported from a study in USA that colistin resistance is reported in 18% isolates of *A. baumannii* recovered from solid organ transplant patients.[22]

The overall frequency of *Acinetobacter* infection in our setup was about 11%. Antimicrobial susceptibility of this pathogen varies significantly among regions/centres. Therefore, local surveillance studies are required to look for the most suitable empirical therapy. Since there are various mechanisms of resistance in *Acinetobacter* spp., for the development of valuable strategies, it is important to comprehend the interplay of various resistant mechanisms.

The main limitation of the study was that it showed results from a single centre and may not represent susceptibility profile of the pathogen at other centres in Pakistan. It was purely laboratory based and there was no clinical correlation to see the therapeutic outcome of the antibiotics used to treat this pathogen. It would be more valuable if multicentre studies are carried out to find out the susceptibility pattern of *Acinetobacter* spp. isolates.

CONCLUSION

Acinetobacter species is an emergent and global nosocomial pathogen. About 11% of soft tissue and wound infections are caused by *Acinetobacter* species in our set up. *Acinetobacter baumannii* is the most prevalent (88%) among *Acinetobacter* spp. Doxycycline was the most sensitive antibiotic to which 45% of the tested isolates were sensitive. Sensitivity to all other antimicrobials was 15% or less. Resistance pattern of *Acinetobacter* spp. is quite alarming in our healthcare settings so effective infection control practices and

judicious use of antibiotics is mandatory, as well as clinical guidance regarding the potential risks for therapeutic failure is imperative.

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