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Major Article

Antimicrobial susceptibility of *Acinetobacter* clinical isolates and emerging antibiogram trends for nosocomial infection management

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Abstract

Introduction: The drug resistant *Acinetobacter* strains are important causes of nosocomial infections that are difficult to control and treat. This study aimed to determine the antimicrobial susceptibility patterns of *Acinetobacter* strains isolated from different clinical specimens obtained from patients belonging to different age groups. **Methods:** In total, 716 non-duplicate *Acinetobacter* isolates were collected from the infected patients admitted to tertiary-care hospitals at Lahore, Pakistan, over a period of 28 months. The *Acinetobacter* isolates were identified using API 20E, and antimicrobial susceptibility testing was performed and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** The isolation rate of *Acinetobacter* was high from the respiratory specimens, followed by wound samples. Antibiotic susceptibility analyses of the isolates revealed that the resistance to cefotaxime and ceftazidime was the most common, in 710 (99.2%) specimens each, followed by the resistance to gentamicin in 670 (93.6%) isolates, and to imipenem in 651 (90.9%) isolates. However, almost all isolates were susceptible to tigecycline, colistin, and polymyxin B. **Conclusions:** The present study showed the alarming trends of resistance of *Acinetobacter* strains isolated from clinical specimens to the various classes of antimicrobials. The improvement of microbiological techniques for earlier and more accurate identification of bacteria is necessary for the selection of appropriate treatments.

Keywords: *Acinetobacter*. Antibiotic resistance. Prevalence. Nosocomial infections. Carbapenems.

INTRODUCTION

Acinetobacter is a genus of Gram-negative coccobacilli, which are non-motile, oxidase-negative, and catalase-positive, and occur in pairs under magnification[1]. *Acinetobacter* species are opportunistic pathogens predominantly found in immunocompromised patients. They are widespread in nature, and regarded as commensal microbes of human skin and respiratory tract, however, they may cause serious infections, such as endocarditis, urinary tract infections, pneumonia, wound infections, meningitis, and septicemia, especially in individuals with impaired host defenses[2]. Infections caused by *Acinetobacter* species are acquired due to hospitalization, mechanical ventilation, respiratory failure, inadequate treatment, previous infection, or antibiotic therapy and catheterization[3]. Higher colonization rates of the throat, and respiratory and digestive tracts are well documented in several previous outbreaks[4] [5].

Acinetobacter species are becoming increasingly resistant to nearly all routinely prescribed antimicrobial agents, including aminoglycosides, fluoroquinolones, and broad-spectrum β-lactams. The majority of strains are resistant to cephalosporin class of antimicrobials, whereas the resistance to carbapenems is increasingly reported[6]. The antimicrobial susceptibility testing showed differences between *Acinetobacter* species, with *Acinetobacter baumannii* being the most resistant strains[7] [8].

The aim of the study was to compare the isolation frequency of *Acinetobacter* species form different clinical specimens and the frequency of antibiotic resistance in different age groups. Antimicrobial susceptibility assays were performed using different antibiotics that are frequently recommended by the clinicians.

METHODS

Hospital and clinical isolates

This study was conducted at the Microbiology Section, Chughtai Lab, Lahore, Pakistan, between January 2012 and

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April 2014. Various clinical samples were collected, including urine, pus, blood, fluid (pleural, pericardial, synovial and peritoneal), wound swab, cerebro spinal fluid (CSF), sputum, central venous pressure (CVP) catheter tip, stool, throat swab, ear swab, tracheal secret, nasal swab, from the tip of Foley catheter, tissue (wound and soft tissues), bronchial washing sample, and samples from endotracheal tube (ETT), and PVC tip.

Isolation and identification of bacteria

The preliminary identification of bacteria was done by standard microbiological procedures[9]. The obtained *Acinetobacter* isolates were further sub-cultured on MacConkey agar and confirmed by API 20NE (Biomerieux, France), according to the manufacturer's instructions.

Antimicrobial susceptibility analyses

Acinetobacter isolates were tested for the susceptibility to ampicillin/sulbactam, cefepime, cefotaxime, ceftazidime, ceftriaxone, imipenem, meropenem, amikacin, gentamicin, tobramycin, doxycycline, tigecycline, ciprofloxacin, levofloxacin, sulfamethoxazole/trimethoprim, piperacillin/tazobactam, colistin, and polymyxin B (300U each). These analyses were performed by standard disc diffusion technique and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[5]. The bacterial suspension of each sample was made and compared with 0.5McFarland turbidity standard. The cartridges containing antimicrobial susceptibility discs (Oxoid, UK) were stored at between 4°C and -20°C, and used after the incubation at room temperature. Mueller-Hinton agar plates were inoculated and incubated at 35°C for 18h, and the zones of inhibition were measured. *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains, according to the CLSI protocol.

Broth dilution method was used to determine the susceptibilities of the isolated strains to tigecycline and colistin. The Food and Drug Administration (FDA), USA, approved breakpoints for the agents against *Enterobacteriaceae* were used to determine tigecycline susceptibility in *Acinetobacter* isolates (resistance, ≥8mg/L; susceptibility, ≤2mg/L). Minimum inhibitory concentration (MIC) breakpoints for colistin were used according to the CLSI guidelines (resistance, ≥4mg/L; susceptibility, ≤2mg/L)[10][11].

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 (Microsoft, USA). The descriptive analysis was performed by calculating frequencies of isolation of *Acinetobacter* and percentage resistance among isolates to various antimicrobial agents.

RESULTS

In total, 716 *Acinetobacter* isolates were obtained (**Table 1**), with the majority from the respiratory samples, followed by the isolates from surgical or burn wounds.

TABLE 1 - Distribution of *Acinetobacter* isolates obtained from different clinical specimens.

	Number	Percentage
Urinary tract infection		
urine	55	7.7
septicemia		
blood	34	4.7
fluid (pleural, pericardial, synovial and peritoneal)	20	2.8
CSF	14	2.0
Surgical/wound infection		
pus	122	17.0
wound swab	76	10.6
ear swab	7	1.0
tissue (wound and soft tissues)	19	2.7
Respiratory samples		
sputum	60	8.4
tracheal secretion	201	28.1
bronchial washing	14	2.0
Catheter tips		
CVP tip	55	7.7
Foley's tip	28	3.9
ETT	11	1.5
Total	**716**	

CSF: cerebro spinal fluid**; CVP:** central venous pressure**; ETT:** endotracheal tube.

Prevalence of *Acinetobacter* resistance according to the age group

The prevalence of *Acinetobacter* strain resistance was shown to be higher in individuals aged between 21 and 50 (**Figure 1**).The frequency of *Acinetobacter* strains isolated alone or in the combination with other bacterial strains isolated from the clinical specimens are different, with 72% of *Acinetobacter* strains isolated alone (519 isolates), as presented in **Table 2**.

Antimicrobial susceptibility profile

Antibiotic resistance profile of *Acinetobacter* isolates was determined using 18 antibiotics, and this revealed that the isolated strains were most commonly resistant to cefotaxime and ceftazidime [for both of them, 710 (99.2%) isolates were shown to be resistant], followed by the strains resistant to gentamicin [670 (93.6%)], and imipenem [651(90.9%)] (**Table 3**). However, most of the tested isolates were susceptible to tigecycline, colistin, and polymyxin B.

TABLE 2 - Percentage of *Acinetobacter* isolated alone, or in combination with other isolates from clinical specimens.

Isolates	Number	Percentage
Acinetobacter only	519	72.0
Candida	43	6.0
Escherichia coli	84	12.0
MRSA	11	2.0
Proteus	11	2.0
Klebsiella	17	2.0
Pseudomonas	24	3.0
Other	7	1.0
Total	**716**	**100.0**

MRSA: methicillin-resistant *Staphylococcus aureus*.

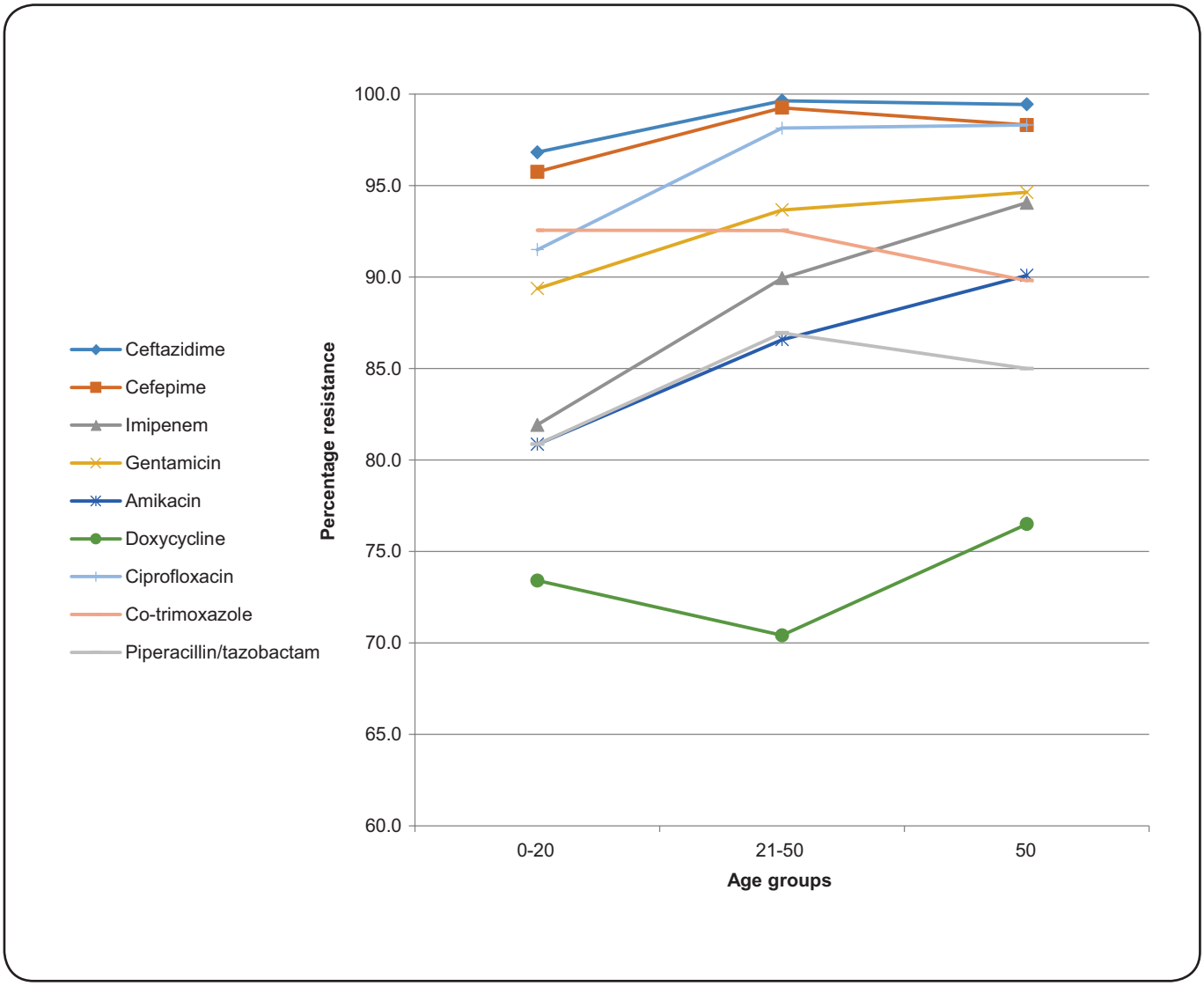

FIGURE 1 - Resistance patterns of *Acinetobacter* isolates to various classes of antimicrobial agents according to the different age groups.

TABLE 3 - Resistance pattern of *Acinetobacter* isolates to various antimicrobial agents.

	Sensitive		Resistant	
Antimicrobials	**n**	**%**	**n**	**%**
Ampicillin/sulbactam	3	0.4	713	99.6
Cefepime	12	1.7	704	98.3
Cefotaxime	6	0.8	710	99.2
Ceftazidime	6	0.8	710	99.2
Ceftriaxone	6	0.8	710	99.2
Imipenem	65	9.1	651	90.9
Meropenem	66	9.2	650	90.8
Amikacin	89	12.4	627	87.6
Gentamicin	46	6.4	670	93.6
Tobramycin	182	5.4	534	74.6
Doxycycline	188	26.3	527	73.7
Tigecycline	711	99.3	5	0.7
Ciprofloxacin	19	2.7	697	97.3
Levofloxacin	20	2.8	696	97.2
Sulfamethoxazole/trimethoprim	63	8.8	653	91.2
Piperacillin/tazobactam	106	14.8	610	85.2
Colistin	714	99.9	1	0.1
Polymyxin B	716	100.0	0	0.0

DISCUSSION

The aim of this study was to characterize *Acinetobacter* samples obtained from the infected patients and the antimicrobial susceptibility of these isolates to various antibiotics commonly used in clinical practice. In total, 716 *Acinetobacter* strains were isolated. The higher isolation rates of *Acinetobacter* from the respiratory samples are in agreement with the results reported previously in other countries[12].

The resistance rates of *Acinetobacter* isolates were 10-51% against amikacin, 0-81% against gentamicin, 0-81% against ceftazidime, 19-81% against ciprofloxacin, 36-75% against piperacillin/tazobactam, and 5-19% against imipenem, as reported in a surveillance study from the intensive care units (ICU) of five European countries[13]. *A. baumannii* strains collected from 11 European countries between 1997 and 2000 were reported to be susceptible to imipenem and meropenem, with resistance rates of 16% and 18%, respectively[14]. However the subsequent data from 12 countries revealed a significant increase in the resistance rates against imipenem (42.5%) and meropenem (43.4%)[15].

The antibiotic resistance data collected around the world demonstrated that the resistance rates of *Acinetobacter* species to imipenem ranged from 0-40% between 2000 and 2004[16]. The prevalence of imipenem resistance in *Acinetobacter* species increased from zero in 1991 to 50% in 2001, as shown in a study conducted in a Spanish hospital[17]. The resistant rates to ampicillin/sulbactam, imipenem, and meropenem were 51.6%, 26.3%, and 29.6%, respectively, of *Acinetobacter* isolates from 30 European centers[18].

Colistin or tigecycline remain the treatment options for the management of most of the cases of infections caused by multi-drug resistant *A. baumannii* strains. We showed that, among the strains we isolated, only 0.7% and 0.1% were resistant to tigecycline and colistin, respectively. This is in accordance with the previous studies[16] [18]. However, the emergence of *Acinetobacter* resistance against tigecycline and colistin is reported with an increasing frequency[18] [19]. In a surveillance study in Europe, the resistance of *A. baumannii* against polymyxin B was shown to be 2.7%[18]. A surveillance study in Greece showed that 3% of *Acinetobacter* strains isolated from ICU patients were resistant to colistin[20]. Furthermore, tigecycline and colistin resistance rates of *A. baumannii* strains isolated in Germany were 6% and 2.8%, respectively[21]. A study in Turkey reported considerably higher tigecycline resistance rates (25%) of *Acinetobacter* strains[22].

Acinetobacter infections are sometimes accompanied by the infections caused by other microorganisms, and here, we showed that these species are mainly C*andida*, *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Proteus*, *Klebsiella,* and *Pseudomonas* species (**Table 2**). These co-infections may increase patient mortality, highlighting the importance of choosing the appropriate antimicrobial therapy[23]. The analyses of antibiotic resistance patterns according to the age groups showed that the number of the strains resistant to most of the antimicrobial agents increased with age, except the strains resistant to co-trimoxazole and piperacillin/tazobactam (**Figure 1**). Some differences in the antibiotic resistance patterns between the urinary tract pathogens isolated from different age groups were observed, however, the rate of resistance to different antibiotics was higher in pediatrics patients compared with that in the middle aged and elderly patients[24]. Another study demonstrated the differences between the age groups in susceptibility patterns among *E. coli* urinary tract isolates for all tested antibiotics except co-trimoxazole[25]. A microarray-based study showed that the antibiotic resistance gene diversity is age-related and that these genes accumulate in the members of human gut microflora starting from infancy, and their interactions become gradually more complex with age[26]. To the best of our knowledge, the differences in the *Acinetobacter* susceptibility to different antimicrobial agents between different age groups have not been reported before.

Taken together, this study identified the differences in the antibiotic resistance of *Acinetobacter* isolates obtained from hospitalized patients in Pakistan. *Acinetobacter* strains have the capacity to acquire antimicrobial resistance rapidly, and therefore, the resistance to even newer antimicrobials is reported worldwide. This allows them to cause nosocomial outbreaks in hospitals. Therefore, decreasing the pace of the emergence of antimicrobial resistance of *Acinetobacter* species is crucial, through the restricted use of antimicrobials, and the enforcement and surveillance of antibiotic Stewardship Programs in health care settings.

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Conflict of interest

The authors declare that there is no conflict of interest.

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