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ANTIMICROBIAL RESISTANCE PROFILE OF *ACINETOBACTER BAUMANNII* IN A TERTIARY CARE CENTRE.

Microbiology

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ABSTRACT

Acinetobacter baumannii is one of the significant pathogens to cause healthcare-associated infection worldwide, mainly infecting critically ill patients. The present study aimed to analyse the antimicrobial resistance of *Acinetobacter baumannii* in a tertiary care hospital. *Acinetobacter baumannii* isolates were identified during one year period in a tertiary care hospital. Antimicrobial susceptibilities were determined for 238 isolates by Kirby-Bauer disk diffusion method and VITEK2-AST cards (Biomérieux, France) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Majority of samples analysed were from inpatient (91.6%) and predominantly from tracheal aspirates. The isolates of *Acinetobacter baumannii* were most resistant to piperacillin (97.61%), ceftazidime (95.71%) and least resistant to colistin (3.26%). The drug of choice in multidrug-resistant strains of *Acinetobacter* spp. are carbapenems and 83.62% isolates of *Acinetobacter baumannii* were resistant to imipenem. There is a high incidence of resistant isolates of *Acinetobacter baumannii* to a majority of antimicrobial classes, including carbapenems. This study will guide the empirical therapy and suggest that surveillance of antimicrobial resistance of *Acinetobacter baumannii* is necessary.

KEYWORDS

Acinetobacter baumannii, antimicrobial resistance

INTRODUCTION

Acinetobacter baumannii is ubiquitous, free-living, aerobic, Gram-negative coccobacilli that prefers a moist environment and can be obtained from soil, water, and sewage.[1] With its persistent resistance to disinfectants, it can live on hospital equipment, including plastics for long periods. It is one of the significant pathogens to cause healthcare-associated infection (HAI) worldwide, mainly infecting critically ill and immunocompromised patients in intensive care units.[2] Antimicrobial resistance among nosocomial isolates of *Acinetobacter baumannii* complicates therapy and adversely affects clinical outcomes and treatment costs.[1,3]

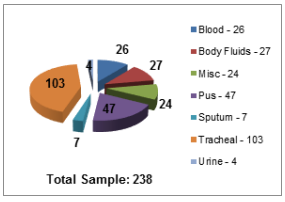
The National Nosocomial Infections Surveillance System reported a significant increase in the proportion of *Acinetobacter* among all Gram-negative aerobes isolated during the seventeen years of the study period (from 1986 through 2003).[4,5] *Acinetobacter baumannii* also has been recognised as a worldwide emerging cause of healthcare-associated infection outbreaks and is listed as one of the six top-priority dangerous microorganisms by the Infectious Diseases Society of America (IDSA).[6,7,8]

The growing number of healthcare-associated infection and rapid increase in antimicrobial-resistant isolates have prompted us to analyse the antimicrobial resistance profile of *Acinetobacter baumannii* isolated from different clinical samples in patients during one year at a tertiary care centre.

MATERIALS AND METHOD

Two hundred and thirty-eight consecutive non-repetitive isolates of *Acinetobacter baumannii* were collected during one year period at a tertiary care hospital. The strains were cultured from Tracheal aspirate (103 or 43.27%), Pus (47 or 19.74%), Body fluids (27 or 11.34%), Blood (26 or 11%), Miscellaneous (24 or 10.08% including wound swabs), Sputum (7 or 2.94%) and Urine (4 or 1.68%). The distribution of samples is provided in Figure 1.

Figure 1: Distribution of *Acinetobacter baumannii* in clinical samples



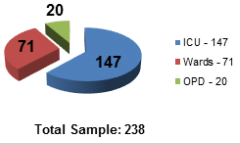
Isolates were identified at the species level with conventional phenotypic methods such as colony morphology, standard biochemical tests, and microbiological processes. They are Gram-negative coccobacilli, strictly aerobic, oxidase negative, nonmotile and catalase-positive, and species differentiation was done based on 10% lactose fermentation, glucose oxidation, gelatin liquefaction, haemolysis, growth at 37°C and 44°C, susceptibility to penicillin and chloramphenicol discs.[9]

All isolates of *Acinetobacter baumannii* were determined for susceptibility to fourteen antimicrobial agents (AMA), twelve antimicrobial agents by Kirby Bauer disc diffusion method and antibiotic sensitivities of isolates for colistin and tigecycline were determined by broth dilution using VITEK2-AST cards respectively, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The Vitek2-AST susceptibility card containing tigecycline at concentrations of 0.75, 2, and 4 µg/ml was used according to the manufacturer's recommendations. The results for tigecycline were interpreted by using the United States Food and Drug Administration (USFDA) recommendations for *Enterobacteriaceae* spp. (≤2 µg/ml), as CLSI 2012 has no guidelines for tigecycline sensitivity for *Acinetobacter baumannii,* whereas the European Committee on Antimicrobial Susceptibility Testing (EUCAST) gives the sensitivity of ≤1 µg/ml for isolates of *Acinetobacter baumannii* in case of tigecycline, but that is considered to be more conservative.[10]

RESULTS

Our microbiology laboratory received a total of 8883 samples during one year period. From a total of 1698 isolates of various bacteria obtained from different clinical specimens, we evaluated 238 (14.01%) isolates of *Acinetobacter baumannii* during the study period. Majority of samples analysed were from inpatient (91.6%) and predominantly from tracheal aspirates, and 147 (61.76%) samples were from ICU patients. The overall percentage of isolation of *Acinetobacter baumannii* in the hospitalised cases was 12.83%, and that of OPD cases was 1.1%. A location-wise distribution of isolates is depicted in Figure 2.

Figure 2: A location-wise distribution of *Acinetobacter baumannii* isolates



The isolates of *Acinetobacter baumannii* were found to be most resistant to piperacillin (97.61%) and ceftazidime (95.71%), whereas 94.68%, 94.65% and 90.67% isolates were resistant to combinations of ampicillin-sulbactam, piperacillin-tazobactam and sulphamethoxazole-trimethoprim respectively. Isolates showed resistance of 91.30% to ceftriaxone. 90.59% isolates were resistant to ciprofloxacin and 65.05% to levofloxacin. The antimicrobial agent of choice in multidrug-resistant (MDR) strains of *Acinetobacter* spp. are carbapenems, but this study showed 83.62% isolates of *Acinetobacter baumannii* to be resistant to imipenem. In the case of gentamicin and tobramycin, a total of 79.91% and 63.71% of isolates showed resistance. The resistance rates of strains for minocycline, tigecycline and colistin were found to be low with 15.21%, 5.91% and 3.26% respectively.

Most of the isolates of *Acinetobacter baumannii* were found to be most sensitive to colistin (96.07%), whereas 76.08%, 73.37% and 30.97% isolates were susceptible to minocycline, tigecycline and tobramycin respectively.

The sensitivity towards imipenem was only 12.06%, and in the majority of strains, the sensitivities to various other antimicrobial agents were less than 10%. The susceptibility pattern of all isolates is depicted in Table 1* and Figure 3#.

DISCUSSION

Acinetobacter spp. is classified in the family *Moraxillaceae*, which includes *Moraxella, Psychrobacter*, and related organisms. [5,11] *Acinetobacter baumannii* is gram-negative coccobacilli, with a DNA G+C content of 39 to 47 mol%, that is strictly aerobic, nonmotile, catalase-positive, and oxidase negative. [9] The negative oxidase test is essential for rapid presumptive identification to differentiate the genus *Acinetobacter* from other similar non-fermentative organisms and members of the *Enterobacteriaceae* family.

Over the last three decades, *Acinetobacter* baumannii has gained importance as a leading HAI causing pathogen, partly due to its impressive genetic capabilities to acquire resistance and partly due to high selective pressure, especially in critical care units. [5] This low-virulence organism has turned into a multidrug-resistant pathogen. *Acinetobacter baumannii* contributes about 80% of all *Acinetobacter* hospital-acquired infection.[5]

Acinetobacter baumannii is an opportunistic pathogen associated with a broad spectrum of diseases including hospital-acquired pneumonia, bacterial meningitis, endocarditis, skin and soft tissue infections, urinary tract infections, acute conjunctivitis, burn wound infections and bacteremia. [1,9] *Acinetobacter baumannii* environmental isolates were capable of surviving for a longer time in tap water, normal saline, and distilled water than respiratory tract isolates with a pH range of 4.5 to 8 and temperature between 18°C to 37°C.[12]

Infections caused by *Acinetobacter* species are associated with adverse clinical outcomes, including high rates of morbidity and mortality, prolonged hospital stay, and substantial health care expenses. During the past decade, increasingly resistant strains of *Acinetobacter baumannii* have emerged, necessitating higher use of broad-spectrum antibiotics, such as imipenem and ampicillin-sulbactam. Of particular concern are the strains of *Acinetobacter baumannii* that are resistant to all commonly used antibiotics and are susceptible only to colistin and tigecycline.[13] In this study also, most of the isolates are resistant to multiple drugs and sensitive to mainly colistin, minocycline and tigecycline.

In the literature, various descriptions have been used to describe the term multidrug-resistant *Acinetobacter baumannii* (MDR-AB).[14] An isolate should be resistant to all of the following thirteen antibiotics: ciprofloxacin, levofloxacin, gentamicin, netilmicin, amikacin, ceftriaxone, cefotaxime, ceftazidime, cefepime, ampicillin-sulbactam, cefoperazone-sulbactam, ticarcillin-clavulanate and piperacillin-tazobactam, before it is counted as an "MDR-AB". Some studies report MDR-AB are as non-susceptibility to three or more classes of drugs. [14,15] In the present study, 36 isolates were resistant to seven antimicrobial agents out of a total of thirteen quoted above by various reports (Isolates were studied for susceptibility to a total of fourteen antimicrobial agents according to CLSI guidelines). On observing the resistance pattern of isolates against these seven antimicrobial agents (ciprofloxacin, levofloxacin, gentamicin, ceftazidime, ceftriaxone, ampicillin-sulbactam, and piperacillin-tazobactam), a total of 36 isolates fit into the criteria of MDR-AB in this centre. [15] Out of these 36, a total of 29 were from ICUs, and seven are from various wards.

The emergence of multidrug resistance microbes showing resistance to β-lactam antimicrobials, aminoglycosides and fluoroquinolones have emerged worldwide due to production of β-lactamases, AmpC-β-lactamases and Extended-Spectrum β-lactamases (ESBLs). [16] In this study, more than 90% isolates of *Acinetobacter baumannii* are resistant to earlier sensitive antimicrobial agents including cephalosporins, fluoroquinolones and various combinations. [17] The most alarming information brought out by this study is that 83.62% isolates were resistant to imipenem, which is relatively in concordance with recent studies done in India, but it is higher as compared to earlier studies done at this centre in isolates of *Pseudomonas aeruginosa* and studies conducted in the same region.[18,19,20] Resistance to carbapenems is often mediated by superbugs due to production of metallo-β-lactamases (MBL). [21] Moreover, the widespread dissemination of the new NDM-1 metallo-β-lactamase strains require particular attention as the genetic background demonstrates extreme mobility and versatility. Carbapenemases are becoming a worrisome global problem, and molecular epidemiological studies will be critical in determining, tracking and better understanding the dissemination of these plasmid-mediated enzymes.[22]

Pathogens that produce carbapenemases along with MBL, ESBL or AmpC-β-lactamases are particularly challenging for clinicians and are a significant threat worldwide. It is an alarming public health concern since carbapenemases containing isolates are highly resistant to most antibiotic classes, which is evident in findings of this study. [22] Carbapenems are the only therapeutic option against this multi-resistant Gram-negative pathogen, but with resistance to them, the only option left for treatment is colistin or tigecycline, which are highly toxic to patients.[23] Colistin and tigecycline have been proven to have an excellent in vitro activity against *Acinetobacter baumannii* pneumonia isolates, even in carbapenem-resistant strains.[8,24]

Presently colistin is the most sensitive antimicrobial against *Acinetobacter baumannii* with a sensitivity of 96.07% which is in line with various other studies. [6] SENTRY Antimicrobial Surveillance from 2001 to 2011, which included different centres from the USA, Europe, Latin America and the Asia-Pacific region, revealed colistin resistance of *Acinetobacter baumannii* remained at a low level (0.9%–3.3%).[6,25]

Acinetobacter baumannii isolates are showing resistance of 5.91% to tigecycline, but the concerning factor is 20.71% isolates in the intermediate zone, whereas isolates showed 15.21% resistance to minocycline. Tigecycline is the first representative of the glycylcycline class of antibacterial agents to be marketed for clinical use. [26] The USFDA have approved its use for complicated intra-abdominal and complicated skin and skin structure infections. Regarding *Acinetobacter baumannii*, this pathogen is susceptible to tigecycline in large-scale microbiological studies, but with higher mortality as compared to colistin in some studies.[24,26] We also included tigecycline in our research, though there is no available minimum inhibitory concentration (MIC) interpretation breakpoints of tigecycline against *Acinetobacter baumannii* according to the criteria of CLSI guidelines, so results were interpreted by using the USFDA recommendations for *Enterobacteriaceae* spp. (≤2 μg/ml), as the EUCAST sensitivity of ≤1 μg/ml for isolates of *Acinetobacter baumannii* in case of tigecycline is considered to be more conservative.[10]

In this study, *Acinetobacter baumannii* showed resistance to multiple drugs, including higher resistance to carbapenems which is a matter of concern. Unfortunately, new and effective antibiotics are currently still not available, so a concerted effort by industry, government and institutions is required to improve the situation. [27] The aetiology behind the emergence of drug-resistant bacteria can have multiple causes including selective pressure from the widespread use of antibiotic agents, so proper application of infection control measures are required, and particularly hand hygiene as well as better antibiotic stewardship in order to slow the development of resistance and to decrease high resistance rates.[27]

CONCLUSION

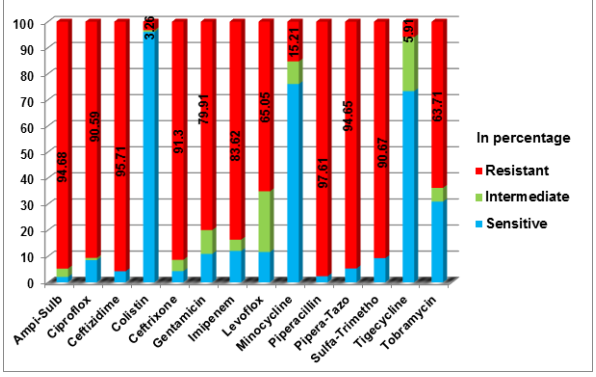
In this study, we concluded that there is a very high incidence of resistant isolates of *Acinetobacter baumannii* to a majority of antibiotic classes, including carbapenems. Most isolates are however

sensitive to colistin. Minocycline and tigecycline are other drugs with reasonable susceptibilities. If *Acinetobacter baumannii* bacteremia is suspected, empiric therapy with these drugs, followed by de-escalation after sensitivity results, is advisable.

Conflict of Interest
None identified

Table 1 Antimicrobial susceptibility profile of *Acinetobacter baumannii*

Antimicrobials	Sensitive	Intermediate	Resistant	Resistant %age
Ampi-Sulbactam	5	8	225	94.68
Ciproflox	20	2	216	90.59
Ceftizidime	10	0	228	95.71
Colistin	228	2	8	3.26
Ceftrixone	10	10	218	91.3
Gentamicin	26	22	190	79.91
Imipenem	29	10	199	83.62
Levoflox	28	55	155	65.05
Minocycline	181	21	36	15.21
Piperacillin	6	0	232	97.61
Pipera-Tazobactam	13	0	225	94.65
Sulfa-Trimetho	22	0	216	90.67
Tigecycline	175	49	14	5.91
Tobramycin	73	13	152	63.71



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