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Antibiogram of *Acinetobacter s*pp. isolated from various clinical specimens in a tertiary care hospital in West Bengal, India.

Bhattacharyya S, Bhattacharyya I, Rit K, Mukhopadhyay PK, Dey JB, Ganguly U, Ray R.

Department of Microbiology, Bankura Sammilani Medical College, Bankura, West Bengal, India .

Abstract

***Acinetobacter* spp. are Gram negative coccobacilli causing various nosocomial infections. They possess different types of Beta-lactamases like SHV, TEM and others which lead to treatment failure in case of infections due to this pathogen. Studying their species distribution in various infections and drug resistance pattern is hence important. Hence our study was aimed to study the different infections caused by *Acinetobacter* spp, in a tertiary care hospital in Eastern India and its antibiotic susceptibility pattern.**

Keywords: *Acinetobacter*, Nosocomial, Resistance.

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Introduction

Acinetobacter spp. are Gram Negative, strictly aerobic, non-fastidious, non-fermenting encapsulated coccobacilli causing mostly nosocomial infections. According to most recent scientific literature, *Acinetobacter* spp. are the second most common non-fermenting Gram negative pathogen isolated from clinical samples after *Pseudomonas aeruginosa* [1]. They are oxidative in metabolism and coccoid in stationary phase of culture [2]. The genus comprises several species, important among which are *A. baumannii* group, *A. lwoffii*, *A. johnsonii*, *A. junii*, *A. hemolyticus* and at least 25 genomospecies [3]. *Acinetobacter* spp. are important causes of device-related infections and urinary tract infections, but in recent years have also been isolated from bloodstream and other sites, and are notorious for resistance to Beta-lactam antibiotics. The spread of Multidrug resistant *Acinetobacter* strains among hospitalized patients has become an increasing cause of concern [4]. Hence it is important to look for novel classes of antibiotics which are effective in treating infection due to *Acinetobacter* spp.

Materials and Methods

The study was conducted from June 2010 to May 2011 in the Department of Microbiology, Bankura Sammilani Medical College, Bankura, West Bengal, India.

One Hundred (100) isolates of *Acinetobacter* spp. were recovered from various clinical specimens, namely pus (23 samples), urine (54 samples), CSF (12 samples), throat swab (4 samples), vaginal swab (1 sample) and unspecified swab samples (6 samples). Only the isolates grown from samples of patients developing various symptoms after admission in different wards of the hospital for more than 48 hours (Nosocomial isolates) were selected for the study. The isolates were speciated and their antimicrobial resistance pattern was studied. The samples received in the laboratory were inoculated on 5% Sheep Blood Agar and Mac Conkey Agar and incubated overnight aerobically at both 37°C (to isolate *Acinetobacter spp*. other than *A. johnsonii*) and 25°C (to isolate *A. johnsonii*, respectively). Thereafter species identification and in-vitro antibiotic susceptibility tests were performed. In case of urine samples, the isolates were subjected to biochemical tests and antimicrobial susceptibility only if the colony count was significant (> 10^5 CFU/ml). *Acinetobacter* spp. were identified by characteristic colonies (Non Lactose-fermenting, glistening, small mucoid colonies), Gram staining pattern and standard biochemical reactions [1] (Catalase, Oxidase, Hugh-Leifson's Oxidation-Fermentation test, Indole production, Citrate utilization, Motility, Urease activity, Reaction in Triple Sugar Iron medium), as shown in Table 1.

After identification by phenotypic methods, antibiotic susceptibility was performed for each isolate by the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar [(4)] using 0.5 MacFarland Turbidity standard and comparing zone sizes with Control strain *P. aeruginosa* ATCC 27853. The following antibiotic discs were used:-

Amikacin (30 µg), Penicillin G(10 µg), Piperacillin-Tazobactum(100/10µg), Cefotaxime(30µg), Ceftazidime (30µg) , Gatifloxacin(10µg) , Levofloxacin(5µg), Ciprofloxacin(5 µg), Cotrimoxazole(25µg), Tetracycline(30µg) (in case of samples other than urine) and Nitrofurantoin (300 µg) (for urine samples only). Susceptibility results were interpreted by measuring the zone diameters produ-

ced and correlating them with the CLSI standards [5]. ESBL production was tested using the Double-Disc approximation method using Ceftazidime and Ceftazidime-Clavulinic Acid discs [6].

Results

Among the 100 isolates, 54% (54 isolates) belonged to the *A. baumannii* group, 44% (44 isolates) were *A. lwoffii* and 2% (2 isolates) were identified as *A. hemolyticus* based on biochemical reactions already mentioned. *A. johnsonii* was not isolated from any of the samples. All the isolates were of nosocomial origin as mentioned before. *Acinetobacter* spp. formed about 17% of all nosocomial bacterial isolates from all clinical samples processed during the study period, as observed from a record of 100 bacterial isolates recovered from various samples during the same period, and about 33.3% of all bacterial isolates from the I.C.U.

Mean age of the patients was 27 years. Gender ratio was 1.46:1 (Male:female). Thus, a slight male preponderance was observed in our study.

Almost all the isolates showed in-vitro resistance to one or more of the antibiotics mentioned earlier. Among the isolates, 29% (29 isolates) were MDR or Multi-Drug resistant (resistance to 3 or>3 different classes of antibiotics) , with 79.3% of total MDR strains belonging to *A. baumannii* group(23 isolates). Among *A. baumannii* isolates, 44% of were MDR ones.

Amikacin resistance was found in 55.5% isolates, while the figures for Penicillin G, Piperacillin-Tazobactum, Cotrimoxazole, Levofloxacin, Ofloxacin, Gatifloxacin, Ciprofloxacin, Cefotaxime, Ceftazidime, Imipenem and Nitrofurantoin were 100%, 18.2%, 71.8%, 21.42%, 58.82%, 6.75%, 52.17%, 75.2%, 80%, 15% and 64.28% respectively. Thus there was less in-vitro resistance to Levofloxacin compared to other quinolones as also to Imipenem, and very negligible resistance to Gatifloxacin. Beta-lactams showed reduced efficacy while Amikacin and Piperacillin-Tazobactum were slightly effective. Approximately 78.5% of all the isolates were ESBL producers, determined as per the double disc approximation method [6].

Discussion

Acinetobacter spp., fast emerging as agents of opportunistic nosocomial infection with evolving drug resistance, have become a real problem in hospital set-up, particularly in the critical care units. Moreover it often shows variable Gram reaction with pleomorphism and may mimic many common Gram positive cocci [7]. The pathogen was previously conferred various names like *Micrococcus calcoaceticus*, *Mima polymorpha* and *Herellea vaginicola.* The genus *Acinetobacter* has undergone significant modification in nomenclature over the last 30 years. The current genus designation, *Acinetobacter* (derived from the Greek “akinetos”, meaning non-motile) was initially proposed in 1954[8]. The genus now comprises about 28 species, which include *A. baumannii*, *A. calcoaceticus*, *A. johnsonii*, *A. junii*, *A. ursingii* and *A. hemolyticus* among others. There are many genomospecies in addition to the species mentioned. *A. calcoaceticus* has been recovered from soil and water samples only and never implicated in clinical disease. Hence the nomenclature *A. calcoaceticus-baumannii* group is no longer acceptable [8]. *Acinetobacter* spp. are mostly implicated in various nosocomial infections like respiratory tract infections, bloodstream infections, wound infections, urinary tract infections, meningitis and rarely keratitis and other infections [8]. According to our findings, the pathogen was mostly isolated from urine(54% , i.e. 54 isolates) followed by pus(23%, i.e. 23 isolates), CSF(12%,. i.e. 12 isolates) and other samples(11%, i.e. 11 isolates). *Acinetobacter* spp. have been implicated in about 10% of all ICU infections in Europe [9]. In our experience, the pathogen constituted 16.9% of all nosocomial bacterial isolates. Infection is facilitated by the ability of the bacterium to colonise hospital equipment and to persist on inanimate surfaces for prolonged periods of time ranging from 3 days to 5 months, and *Acinetobacter* spp. can be detected on various equipment including bedrails, curtains, ventilation equipments (e.g. AMBU bags, Ventilation filter) [8]. Colonisation of patients, health care workers and healthy individuals occurs frequently. Several virulence factors like lipases and siderophores have been studied [10]. Management of *Acinetobacter* infections is a huge challenge because of the broad array of antimicrobial resistance and the pathogen’s ability to develop new resistance rapidly. Different resistance patterns have been found even with proven clonal isolates of *Acinetobacter* spp. in the nosocomial setting. Antimicrobial agents that are typically active against the pathogen include the Carbapenems(Imipenem and Meropenem), Amikacin, Sulbactum, Colistin, Rifampin and Tetracyclines. Combination therapy can be considered, but is controversial due to no proven improvement in mortality and increased toxicity [8]. *Acinetobacter baumannii* strains inherently possess chromosomally encoded AmpC cephalosporinases that mediate resistance to Cephalosporins. Aminoglycoside-modifying enzymes are highly prevalent in multi drug-resistant *A. baumannii* strains. Resistance to Quinolones is mediated by modifications in DNA gyrase or Topoisomerase IV while that to Tetracyclines occurs via efflux pumps or Ribosomal protection [7]. Resistance has been associated with an 86 kb chromosomal region or resistance island, that is responsible for production of resistance to a large number of antimicrobial agents [7]. Multidrug resistance(MDR), i.e. resistance to Cefotaxime, Cef

tazidime, Amikacin and Ciprofloxacin [11], is an emerging problem with *Acinetobacter* spp. Pan drug resistant *A. baumannii* isolates, i.e. isolates resistant to all antimicrobial agents in-vitro, have been reported from Asia and the Middle-east [8]. In our study, 29% of the isolates were Multi-drug resistant. About 44.4% of *A. baumannii* isolates were multidrug resistant. Studies have quoted isolation of Multi-drug resistant *Acinetobacter* spp. from Indian and Asian hospitals. In a review comparing hospitals of 10 Asian countries, 1.2-87% of all *Acinetobacter* isolates from patients with Hospital Acquired Pneumonia(HAP) were MDR, with MDR strains most prevalent in India and Thailand [12].

In a study from Pune, about 48% to 68.6% *A. baumannii* isolates were MDR[13]. In a report from 48 European hospitals from 2002 to 2004, 32.4%, 34% and 47.6% isolates showed susceptibility to Ceftazidime, Ciprofloxacin and Gentamicin respectively[9]. In Asia and the Middle-east, rates of non-susceptibility are about 40% for Ceftazidime, 35% for Amikacin and 45% for Ciprofloxacin[9]. We found the corresponding figures for these groups of antimicrobials to be 80%, 55.5% and 52.17% respectively. Thus in our study, slightly higher values were recorded for third generation Cephalosporins and Ciprofloxacin respectively. *Acinetobacter* spp. constituted about 33.3% of all bacterial isolates and 25% of all microbial isolates from I.C.U. samples, according to our study.

Colistin, also referred as Polymyxin E, typically retains activity against *Acinetobacter* spp. in the face of broad-spectrum antimicrobial resistance. However, it can be nephrotoxic and ototoxic which limits its routine use, though the toxicity has been found to be similar to other antimicrobials in the ICU set-up [8].

Notably, our findings show that Levofloxacin is effective against *Acinetobacter* spp.(21.42% resistance), while 6.25% isolates were resistant to Gatifloxacin. Another study has reported Levofloxacin resistance in *Acinetobacter baumanii* in the order of 26% [14]. Thus Levofloxacin, a S-enantiomer of Ofloxacin, being safer and better tolerated than other fluoroquiniolones [15], can be a lesser toxic and cost-effective therapeutic option against *Acinetobacter* isolates, especially in this part of India. Carbapenem resistance is an emerging problem with *Acinetobacter* spp. Only a few centres like I.M.S., B.H.U., Varanasi and A.I.I.M.S.,New Delhi, have mentioned Meropenem resistance in *Acinetobacter* spp. to be about 6.4% and 22.16% respectively in their studies[16,17]. One report from the U.S.A has quoted Imipenem resistance in *Acinetobacter baumannii* in the order of 23.1% in their study[18]. A similar study from India mentions that about 14.2% of *A. baumannii* isolates in their study were Imipenem resistant [19]. *Acinetobacter* spp., in general show greater in vitro resistance to Meropenem than Imipenem [16,17]. Our results show that about 15% of all isolates of *Acinetobacter* spp. were resistant to Imipenem. Another important finding of our study is that ESBL production was found to be of the order of 78.5%, which is in concordance with results from a study in Meerut, North India which has shown this figure to be about 75% [20]. However, studies from Bangalore, South India, have reported a low prevalence of ESBLs in *Acinetobacter* spp.(28%) [21]. There are only a handful of reports in the literature regarding the prevalence of ESBLs in *Acinetobacter* spp. from India, especially Eastern India, and our study thus merits mention.

Limitations of the study

The sample size (100)was not very large and hence the species distribution as mentioned here needs to be correlated with studies taking large number of isolates. Also, the in-vitro resistance of *Acinetobacter* spp. to Colistin was not studied, especially in the I.C.U., which could be epidemiologically important.

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Correspondence to: *Bhattacharyya S*