Isolation and speciation of Acinetobacter species from various clinical samples and their antibiogram

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Abstract

Acinetobacter species are Gram negative nonfermentative bacteria commonly present as free living saprophytes. Acinetobacter has emerged as an important nosocomial pathogen involved in outbreaks of hospital infections. The ubiquitous organism has been recovered from hospital environment, from colonized or infected patients or from staff. The emergence of multi drug resistance poses a significant challenge in identifying and treating this organism. Retrospective study was carried on clinical samples received from various departments. Acinetobacter isolates were identified and speciation was done by using biochemical methods and antibiotic sensitivity pattern of these isolates was studied.

A total of 210 Acinetobacter species were isolated from various clinical samples, commonest being urine samples followed by sputum, blood, pus samples. The most common species isolated were: A.baumanii (79%), A.iwoffi (17%), A.haemolyticus (2%) and A.junni (2%). Resistant pattern to various drugs was Amoxyclauv 83.6%, Imipenam 48.3%, Piperacillin-tazobactum 42.6%, Amikacin 58%, Gentamicin 66%, Ceftazidime 72%, Cefotaxime 68%, Ciprofloxacin 76%. In the present study, the prevalence of Acinetobacter from various samples and its antimicrobial susceptibility pattern was studied. Increased emergence of multi drug resistant strains is an important health concern as it poses a challenge in treatment. A surveillance study is required to know about the strains prevailing in the particular geographical locations and its antimicrobial sensitivity pattern.

Keywords: Acinetobacter, nosocomial pathogen, multi drug resistant strains, intensive care units (ICU), Speciation.

Introduction

Acinetobacter species are aerobic, non-fermenting Gram negative bacteria as they do not utilize carbohydrate as source of energy. They are commonly present as free living pathogen[4]. The ubiquitous organism has been recovered from sewage, soil, water, hospital environment, from colonized or infected patients or from staff[13]. This ubiquitous organism has now recently emerged as a important nosocomial pathogen causing various infections in the hospital setting. Acinetobacter is recognised as one of the ESKAPE pathogens causing nosocomial infections because of its property of being stable in extremes of environmental conditions like temperature, pH, humidity and resistance to commonly used disinfectants[16].

Previously infection due to Acinetobacter was considered as non pathogenic, but in the recent era, it is considered as primary pathogen with special mention to Hospital Acquired Infections(HAI)[3]. It is known to cause most commonly respiratory tract infection followed by urinary tract infections, surgical site infections, endocarditis, meningitis, peritonitis and septicaemia[14]. Intrinsic resistance nature of Acinetobacter has become a troublesome situation in hospital as it poses a difficulty in treatment due to its resistance to various antibiotics. Resistance in Acinetobacter is ascribed due to combination of selective pressure due to use of broad-spectrum antibiotics and dissemination of drug resistant strains among patients[12]. Resistance rates differ from region to region based on various etiologic factors such as weather, antibiotic usage, hospital environment resistant strains and infection control practices.

Infections due to Acinetobacter are rising in hospital environment because of the characteristics like intrinsic resistance, acquired drug resistance and capability to survive in extremes of temperatures. It has become a important pathogen causing nosocomial infections. Since two decades, the emergence of Acinetobacter infections is increasing of which the emergence of multidrug resistant strains is of global concern[17]. Outbreaks in ICUs with multi drug resistant Acinetobacter have been due to presence of indwelling devices[6,23]. Old age patients, patients with underlying chronic diseases are at more chances of developing these infections[9]. The mortality and morbidity rate range from 27% to 91% among immunocompromised in the last three decades[22].

In the current situation, globally opportunistic infections due to Acinetobacter are responsible for 20% infection in ICUs[8]. Although Acinetobacter can cause infection in hospital setting, it can cause infection even in community setting, among healthy individuals, following a calamity[23]. Management of nosocomial infections in a health care setting remains a challenge. A thorough knowledge about the most common species prevailing in particular geographical and its sensitivity pattern are required to combat against these nosocomial infections. Hence, the aim of this study was to isolate Acinetobacter species from various clinical sample and to study its antimicrobial susceptibility pattern.

Material and Methods

A retrospective study was carried out in a tertiary care hospital, VIMS, Ballari for a period of one year from January 2019 to December 2019, after obtaining approval from Institutional Ethics Committee. Various clinical samples from both in patients and out patients were collected aseptically and processed.

Urine samples were inoculated into CLED agar, other samples were inoculated onto MacConkey agar and blood agar. Isolates were conventionally identified by Gram stain, non-lactose fermenting colonies catalase test and oxidase test. Various biochemical tests were used to identify genus Acinetobacter like indole test, citrate test, urease test, mannitol motility medium test and triple sugar iron agar test. Speciation of Acinetobacter was performed on the basis of Hugh and Leifson oxidative-fermentative test (O-F) for glucose, sucrose, lactose, mannitol, gelatin liquefaction, beta hemolysis on blood agar media, nitrate reduction test, urease hydrolysis test (Christensen), decarboxylation of arginine, lysine and ornithine[10].

Antibiotic susceptibility was performed by the Kirby Bauer disc diffusion method according to CLSI guidelines. Antibiotic sensitivity tests were put up for aminoglycosides (Amikacin 30mcg), gentamycin (10mcg), cephalosporins (Ceftazidime 30mcg, Cefotaxime 30mcg), fluoroquinolones (Ciprofloxacin 5mcg), carbapenem (Imipenam 10mcg), Piperacillintazobactam (100mcg/10mcg) and amoxy clauv (20/10mcg). All isolates of Acinetobacter resistant to three or more classes of antibiotics were considered as multidrug resistant (MDR).

Results

During the study period, the total number of samples tested positive for Acinetobacter species was 210 out of 8653 samples received in laboratory. Acinetobacter infection was more common in males (64.2%) compared to females (35.8%) especially in elderly age group. The isolates were predominantly recovered from urine (40%) followed by respiratory tract samples (21%), blood and other samples.

Out of 210 isolates, 166(79%) were identified as *A.baumanii* and remaining 44(21%) were identified as other Acinetobacter species. Among 44 isolates 36(17.14%) were identified as *A.lwoffii*, 5(2.3%) were identified as *A.hemolyticus* and 3(1.4%) were identified as *A.junni*.

Antimicrobial susceptibility testing was performed as per CLSI guidelines. The resistance pattern to each class of drugs is depicted in fig. 2. Around 83% of the isolates were resistant to amoxy clavunate, followed by ciprofloxacin (76%), ceftazidime (72%) and cefotaxime (68%). Around 66% of the isolates were resistant to gentamicin followed by amikacin (58%). 48.3% of the isolates were resistant to Imipenam and 42.6 5 of the isolates were resistant to Piperacillin tazobactum. Around 74.6% of the isolates were multi drug resistant.

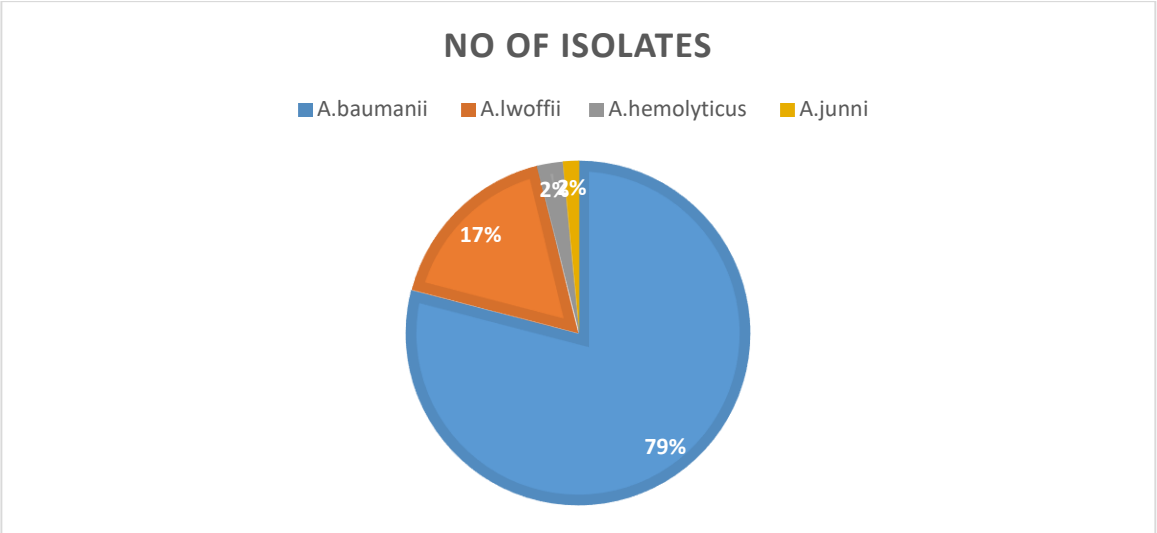

Fig. 1: Distribution of acinetobacter species

Table 1
Distribution of acinetobacter species from clinical samples

Samples	Isolates
Urine	86(40%)
Respiratory tract samples	46(21%)
Blood	35(16%)
Pus	32(15%)
Others	11(5%)

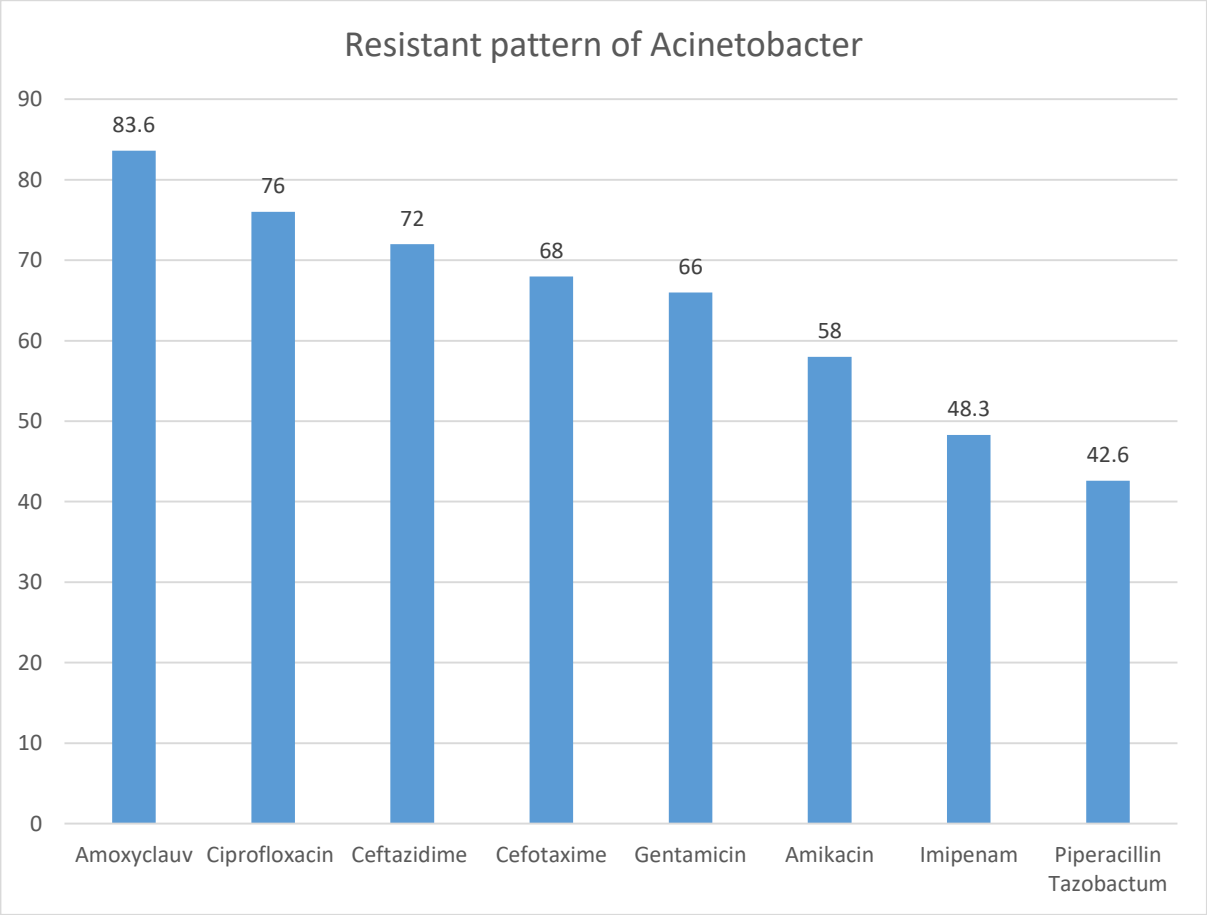

Fig. 2: Resistant pattern of Acinetobacter

Discussion

In the current scenario, Acinetobacter has emerged as an important nosocomial pathogen exhibiting multi drug resistance to various class of antibiotics. The main purpose of the study was to isolate the Acinetobacter species from different clinical samples and to identify its antimicrobial susceptibility pattern. Isolation of Acinetobacter species was 2.4% which was similar to a study by Sharma et al[19] where the isolation rate of Acinetobacter species was 6.4%. In the present study, infection was more common in the age group 40-60 which was similar to a study carried out by Gupta et al[5]. The infection was more common in males compared to female which was similar to a study by Mohamad et al[11].

In the present study, Acinetobacter species was isolated more commonly from urine sample followed by respiratory tract samples which were similar to study conducted by Tewari et al[21]. Isolation of Acinetobater species from blood was 16% which was similar to a study by Tewari et al[21], although few studies have reported isolation rates from 7-25%[7]. The rate of isolation from different clinical samples is mainly predisposed by the differences in the sample types, previous usage of antibiotics, infection control practices. Around 35.6 of isolates were isolated from ICU which was similar to a study by Mohamad et al[11]. 30 % of the isolates were isolated from ICU in contrast to a study by Tahseen et al[20]. This difference in the variation could be due to different geographical setting and hospital environment.

Among the isolates, *A.baumani* is most commonly followed by *A.lwoffi, A.haemolyticus, A.junni*, similar to studies of Gupta et al[5] and Fayyaz et al[2].

The main problem with Acinetobacter species is multi drug resistant nature. This multi drug resistance varies according to different geographic locations. In the present study around 74.6% of the isolates were multi drug resistant, resistant to more than 3 classes of drugs. The result was similar to a study by Tewari et al[21] and Bansal et al[1]. In the present study, around 76% of the isolates was resistant to ciprofloxacin which was similar to a study by Kaur et al[8]. In the present study, approximately 62 % of the isolates were resistant to aminoglycosides almost similar to a study by Rajakumari et al[15].

Around 48% of the isolate were resistant to carbapenams. The difference in the resistance rate could be due to different geographical locations and various other etiological factors. In the present study, resistance to colistin could not be detected. The overall incidence of Acinetobacter in Intensive Care Unit was around 35.6%. The sensitivity pattern of Acinetobacter strains varies among different regions. In order to minimize the infections due to Acinetobacter species, surveillance studies are required to identify the strains prevalent in particular geographical location and its antimicrobial sensitivity pattern to initiate empirical treatment when required.

Conclusion

Higher rates of isolation of *Acinetobacter* from various samples indicate its role as nosocomial pathogen and also as an etiological agent in community acquired infection. The emergence of antibiotic resistance leads to therapeutic difficulties. Simplified identification scheme and antimicrobial susceptibility testing provide cost effective approach for speciating *Acinetobacter* species. In developing countries like India, keeping in view of nosocomial pathogen, further studies are required to combat against these species.

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