ACINETOBACTER SPECIES: PHENOTYPIC CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE

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Abstract

Background: *Acinetobacter species* is ubiquitous, aerobic gram-negative coccobacilli that are now emerging as an important nosocomial pathogen.

Objectives: The present study was designed to know the prevalence of Acinetobacter in various clinical samples, their characterization and their antibiotic susceptibility pattern in B.P. Koirala Institute of Health and Sciences, Dharan.

Methodology: Hundred *Acinetobacter* isolates obtained from different clinical specimens were taken. Identification to species level was done according to standard microbiological methods. Antimicrobial susceptibility to 10 antimicrobial agents was performed by Kirby Bauer method with special reference to Minimum inhibitory concentration to meropenem.

Result : The predominant *Acinetobacter* isolate was *Acinetobacter calcoaceticus (*42%) followed by *Acinetobacter baumannii (34%) , Acinetobacter lwoffii (18%)* and *Acinetobacter junii (6%).* Resistance pattern to various drugs were Meropenem (19%), Piperacillin (96%), Piperacillin-tazobactum (43%) , Amikacin (51%), Ceftazidime (84%), Ceftriaxone (66%), Co-trimoxazole (58%), Gentamicin (57%), Ciprofloxacin (55%), Tetracycline (53%) . Eleven isolates of *Acinetobacter* were resistant to Meropenem as detected by MIC testing whereas resistant *Acinetobacter* by disc diffusion technique were 19 in number.

Conclusion: Occurrence of *Acinetobacter* in our hospital as an important clinical isolate is a serious matter of concern. Moreover, its involvement in wide spectrum of diseases and development of resistance to commonly used antimicrobials has further worsened the situation. Prudent use of antimicrobials, effective surveillance of antimicrobial resistance and adherence to infection control practices, perhaps are the key factors that may prevent the development and dissemination of resistance among the local isolates.

Key words: ***Acinetobacter, infections, resistance***

Introduction

Members of the genus *Acinetobacter* have been implicated in a wide spectrum of infectious diseases. Although associated primarily with nosocomial infection, it has also been involved in cases of community acquired infection.[1] *Acinetobacter* causes mild to severe illness. The number of nosocomial infections caused by *Acinetobacter* species has increased in recent years and is of increasing concern in critically ill patients and the risk factors for this infection are not well established.[2] Its ubiquitous presence, survival ability and rapid development of resistance to the commonly used antimicrobials are responsible for emergence of this organism as a significant nosocomial and opportunistic pathogen. [3]

Acinetobacter infections have been reported for almost all organ system. It is usually an opportunistic pathogen as evidenced by the fact that 14% to 62% of infections are mixed infections. The most common sites involved are the respiratory and urinary tracts and is common among residents of hospitalized patients.[4] In the 1990 to 1992 national nosocomial infections surveillance data, 2% of blood stream infections and 4% of nosocomial pneumonia cases were due to *Acinetobacter.*[4]

Knowledge of the distribution of various species in relation to the variety of infection in hospital setup and to their antimicrobial profile is of utmost importance for effective management of infection caused by the pathogen.

Therefore the study was undertaken to characterize the clinical isolates of *Acinetobacter* upto species level and to study its antimicrobial susceptibility pattern to commonly used antimicrobials in BP Koirala Institute of Health Science hospital.

Materials and Methods

This study was carried out in the Microbiology unit of Clinical laboratory service (CLS) of B.P. Koirala Institute of Health Sciences hospital from June 2008 to May 2009. One hundred *Acinetobacter* isolates obtained from blood, pus, urine, corneal scrapings, sputum, aspirates, cerebrospinal fluid, high vaginal swab, catheter tip, peritoneal fluid, wound swab, tissues were taken.

All the samples were subjected to gram

staining first except blood and urine. Other clinical specimens were inoculated onto blood and MacConkey agar and incubated at 37^0C for 24-48 hours. Urine was plated onto Cysteine lactose electrolyte deficient medium. For the blood samples, brain heart infusion broth was used as primary culture medium. After inoculation of the broth incubation, subculture was done on blood agar and MacConkey agar. And was incubated at 37^0C for 24 hours. [5]

Preliminary identification of *Acinetobacters* was done by the Gram stain findings, testing for motility and the oxidase reaction in all the samples.[5] Non-fermenting gram-negative bacilli that were oxidase-negative and non-motile were identified as *Acinetobacter* spp. All the isolates of *Acinetobacter* was identified to species level by using standard microbiological methods which included reaction on triple sugar iron agar, reaction on Sulphite indole motility, urease production, citrate utilization, hemolysis of sheep blood agar, gelatin hydrolysis and growth at 41^0C and 37 ^{0}C.[6]

Antimicrobial susceptibility of all isolates was determined by the standard Kirby Bauer disk diffusion method according to norms of Clinical Laboratory Standards Institute (CSLI). Antibiotics included were Amikacin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5µg), Co-trimoxazole (1.25/23.75 µg), Gentamicin (10 µg), Tetracycline (30 µg), Meropenem (10 µg), Piperacillin (100 µg), Piperacillin-tazobactam (100\10µg). Further in vitro susceptibility was determined for meropenem by Minimum Inhibitory Concentration (MIC) with agar dilution method and results were interpreted according to CLSI guidelines. Quality control of susceptibility testing was done by using *Pseudomonas aeruginosa (*ATCC 27853).[7]

Results

Out of 100 isolates of *Acinetobacter*, 42 were identified as *Acinetobacter calcoaceticus*, 34 as *Acinetobacter baumannii*, 18 as *Acinetobacter lwoffi* and 6 as *Acinetobacter junii*. Sixty six isolates were obtained from patients admitted to the various wards, 14 from emergency department and 20 from outpatient department.

Among the various specimens from which the isolates were obtained, the highest number was from blood (25) followed by urine (17), device (15), pus (15), wound swab (15), sputum (5), high vaginal swab (3), cerebrospinal fluid (3), CAPD fluid (1) and throat swab (1).

Out of 10 antimicrobials tested the isolates were most resistant to piperacillin (96%) and least resistant to meropenem (19%). Antimicrobial susceptibility pattern by Kirby Bauer disc diffusion method exhibited by *Acinetobacter* is depicted in Table 1.

MIC of Meropenem

Of the 42 isolates of *Acinetobacter calcoaceticus*, 35 were within or below the susceptible breakpoint for meropenem whereas six were above resistance breakpoint. One fell in the intermediate zone. Similarly 28 isolates of *Acinetobacter baumannii* were within the susceptible breakpoint, one was in the intermediate zone and five were above the resistance breakpoint. For all 18 isolates of *Acinetobacter lwoffi* and six isolates of *Acinetobacter junii,* MIC of meropenem was within the susceptible breakpoint.

Discussion

In the last two decades,

Acinetobacter has emerged as a worldwide problem as an important pathogen in hospitalized patients.

Acinetobacter species are often multidrug resistant and associated with life-threatening infections especially in patients with factors that impair normal host resistance.[8] In the present study, 100 isolates of *Acinetobacter* were studied for the characterization and antimicrobial susceptibility. Majority (66 %) were obtained from the admitted patients whereas 14 % were from emergency department and 20 % from outdoor patients.

In our study, 54% of the affected patients were male. Similar pattern was observed in a study conducted in a tertiary care hospital in South India in which male patients formed 58%.[2] *Acinetobacter* species is a heterogeneous group of organisms which is ubiquitous in nature.[9] Greater exposure of male to the external environment as compared to female perhaps plays a role in male predominance in acquisition of infections.

Maximum number of *Acinetobacter* isolates were from blood (25%). Most of our blood isolates were from the cases of neonatal sepsis. Low birth weight, previous antimicrobial therapy, mechanical ventilation, parenteral nutrition and prolonged hospitalization are the known risk factors for bacteremia in such patients.[9]

An attempt was done to identify all the isolates of genus *Acinetobacter* upto species level in this study. Four species were encountered at varying frequencies. *A. calcoaceticus* (42%) was the predominant species, followed by A. *baumannii* (34%), *A. lwoffi* and *A. junii* formed 18% and 6% respectively. This finding is similar to the report published by Communicable Disease Report Weekly .[10] Pedersen et al in 1970 isolated *Acinetobacter antitratus* in 72 cases, *Acinetobacter lwofii* in 42 cases.[11] Smego *et al* in 1985 found that 16/25 isolates of *Acinetobacter anitratus* to be hospital acquired and disease associated and *Acinetobacter lwofii* in only two cases of bacteremia that was also community acquired.[12]

Acinetobacters are known to possess a low potential for virulence. It is their resistance to various antimicrobials, that limits the selection of appropriate drugs for the effective management, thus allowing them to establish

themselves as a difficult organism to control and treat.[13]

Multidrug resistance is well documented phenomenon in clinical strains of *Acinetobacter*.[14] In our study Amikacin and Meropenem showed maximum level of activity with susceptibilities of 49% and 71% respectively. This susceptibility pattern conforms to the recent introduction of these antibiotics in the hospital where the present study was carried out. Increasing resistance to Cephalosporins was observed in our study. The rate of resistance to ceftazidime and ceftriaxone were 87% and 91% respectively. The trend towards resistance to expanded-spectrum cephalosporins was also demonstrated by Joly-Guillou et al and seemed to be related to the presence of cephalosporinases.[15] However despite such resistance , combination therapy using a third generation Cephalosporin and Amikacin could be the best choice for treating *Acinetobacter* infections in our set up.

In the present study resistance to meropenem was determined both by disc diffusion and agar dilution method. By agar dilution method, MIC of eleven isolates of *Acinetobacter* were within the resistant breakpoints, MIC of eighty seven were within the susceptible breakpoints and MIC of two were above the susceptible but below the resistant breakpoint. The isolates found to be resistant by disk diffusion method were found to have MIC range within the susceptible and intermediate breakpoints. Out of nineteen isolates resistant to meropenem by disc diffusion method, seven isolates of *Acinetobacter* had MIC value ranging from 1-4 µg/ml which is below the recommended MIC breakpoint for resistant isolates and one isolate had MIC value 8 µg/ml.

A report from France showed that 17% of *Acinetobacter* spp. was resistant to meropenem by the agar dilution method.[16] Our results were similar to their observation. Low-level resistance to carbapenems as determined by MIC has been reported in several studies.[17,18] In a study by Weinbren et al, isolates were detected to be resistant to carbapenem by disk diffusion method and revealed MICs of 0.5-2 µg/ml, which is below the recommended MIC breakpoint for resistant isolates.[18] In the study done by Sinha and Srinivasa majority of the isolates resistant to meropenem by disk diffusion method were found to have MICs in the sensitive range which is similar to our study.[19]

Conclusion

It can be concluded from the study that *Acinetobacter calcoaceticus* was the species responsible for majority of *Acinetobacter* infection in the hospital under study. The increasing trends towards antibiotic resistance reflect the extensive usage of antibiotics in hospitals which in turn exerts selective pressure on *Acinetobacter* in hospital environment. The infections caused by these organisms are becoming difficult to treat day by day. Effective management and control of *Acinetobacter* infection does not appear to have a simple answer. Perhaps what is essentially required at this point of time is a multifaceted approach comprising of adherence to good infection control practices, prudent use of antimicrobials and continuous monitoring and surveillance of antimicrobial resistance.

References

1. **Jolly-Guillou ML**. Clinical impact and pathogenicity of *Acinetobacter*. Clinical microbiology and infections. 2005; 11:868-73.

2. **Prashanth K, Badrinath S**. Nosocomial infections due to *Acinetobacter* species: Clinical findings, risk and prognostic factors. Indian Journal of Medical Microbiology.2006; 24:39-44.

3. **Towner KJ.** Clinical importance and antibiotic resistance of *Acinetobacter* spp. Journal of Med. Microbiol. 1997; 46: 721-746.

4. **Nerad JL, Seville MT, Snydman DR.** Miscellaneous Gram-Negative bacilli: *Acinetobacter*, *Cardiobacterium*, *Actinobacillus*, *Chromobacterium*, *Capnocytophaga* and others. In: Gorbach SL, Bartlett JG, Blacklow NR. Infectious diseases.2nd ed. WB Saunders Company; 1998.p.1871-1887.

5. **Winn Washington, Allen S, Janda William, Koneman Elmer, Procop Gary, Schreckenberger Paul, Woods Gail.** Koneman's Color Atlas and Textbook of Diagnostic Microbiology. The Nonfermentative Gram-Negative Bacilli. Part II: Taxonomy, Biochemical, Characteristics and Clinical significance of Medically Important Genera of Nonfermenters. Lippincott William and Wilkins. 2006; 316-391.

6. **Baron EJ, Peterson LR, Finegold SM, eds.** Nonfermentative Gram-negative bacilli and coccobacillli. In : Bailey and Scott's Diagnostic Microbiology . 9th ed. Mosby' 1994:386-405.

7. **National Committee for Clinical Laboratory Standards.** 2002.Performance standards for antimicrobial disk susceptibility tests, 7thed. Approved standard M2- A7. National Committee for Clinical Laboratory Standards, Wayne, PA.

8. **Lone R, Shah A, Kadri SM, Lone S, Shah F.** Nosocomial multidrug resistant *Acinetobacter* infections-Clinical Findings, Risk Factors and Demographic Characteristics. Bangladesh J Med Microbiol 2009; 03: 34-38.

9. **Kurcik-Trajkovska B.** *Acinetobacter* spp. – A Serious Enemy Threatening Hospitals Worldwide. Macedonian Journal of Medical Sciences. 2009; 2:1-6.

10. **Communicable Disease Report**. Bacteraemia and bacterial meningitis, England and Wales: weeks 92/30 – 33.1992; 2:154-158.

11. **Pedersen MM, Marso E, Picket MJ.** Non fermentative bacilli associated with man III pathogenecity and antibiotic susceptibility. *Am J Clin Pathol* 1970; 54: 178.

12. **Smego RA.** Endemic nosocomial *Acinetobacter calcoaceticus* bacteremia clinical significance, treatment and prognosis. *Archives Internal Med* 1985; 145: 2174-79.

13. **Gaur A, Bhattacharjee A, Anupurva S, Prakash P, Sen MR, Mohapatra TM**. Clinical significance and epidemiology of multidrug resistant *Acinetobacter* species isolated from nosocomial infections: An experience of a Tertiary care hospital in North India. Indian. J. Prev. Soc. Med.2007.

14. **Pardesi KR, Yavankar SP , Chopade BA**. Plasmid distribution & antimicrobial susceptibility patterns of *Acinetobacter* genospecies from healthy skin of a tribal population in western India. Indian J Med Res . 2007; 79-88.

15. **Seifert H, Baginski R, Schulze A, Pulverer G.** Antimicrobial susceptibility of *Acinetobacter* species. Antimicrob Agents Chemother 1993; 37: 750–753.

16. **Aubert G, Guichard D, Vedel G**. In-vitro activity of cephalosporins alone and combined with sulbactam against various strains of *Acinetobacter baumannii* with different antibiotic resistance profiles. J Antimicrob Chemother. 1996; 37:155-60.

17. **Afzal-Shah M, Livermore DM.** Worldwide emergence of Carbapenem-resistant *Acinetobacter* spp. J Antimicrob Chemother 1998; 41:576-7.

18. **Weinbren MJ, Johnson AP, Kaufmann ME, Livermore DM.** *Acinetobacter* spp. isolates with reduced susceptibilities to carbapenems in a UK burns unit. J Antimicrob Chemother 1998; 41:574-6.

19. **Sinha M, Srinivasa H**. Mechanisms of resistance to carbapenems in meropenem- resistant *Acinetobacter* isolates from clinical samples. Indian Journal of Medical Microbiology.2007;25:121-125

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Antimicrobial susceptibility

Antimicrobial susceptibility pattern by Kirby Bauer disc diffusion method exhibited by *Acinetobacter* is depicted in Table 1.

Table 1: Antimicrobial susceptibility pattern of *Acinetobacter* isolates

Species	No. of isolate	Ak			Ca			Ci			Cf			G			Mr			Pc			Pc-tz			T			Co		
		S	P	R	S	P	R	S	P	R	S	P	R	S	P	R	S	P	R	S	P	R	S	P	R	S	P	R	S	P	R
A.calcoaceticus	42	18	-	24	4	1	37	2	13	27	11	3	28	15	-	27	28	4	10	-	1	41	13	2	20	11	5	26	13	-	29
A. baumannii	34	10	-	24	3	-	31	3	5	26	12	-	22	8	-	26	22	4	8	1	-	33	14	2	18	9	5	20	10	-	24
A.lwoffi	18	16	-	2	5	1	12	3	6	9	13	2	3	16	-	2	16	1	1	1	1	16	15	-	3	11	3	4	14	-	4
A.junii	6	5	-	1	1	1	4	1	1	4	4	-	2	4	-	2	5	1	-	-	-	6	3	1	2	3	-	3	5	-	1

Abbreviations: Piperacillin - tazobactam (Pc-tz), Piperacillin (Pc), Ceftazidime (Ca), Ceftriaxone (Ci), Meropenem (Mr), Gentamicin (G), Amikacin (Ak), Tetracycline (T), Ciprofloxacin (Cf), Trimethoprim-sulfamethoxazole (Co).