Acinetobacter spc. : A major isolates of nosocomial infection's – clinical significance and antimicrobial susceptibility.

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Abstract:
Acinetobacter spp. is ubiquitous, aerobic gram-negative coccobacilli that are now emerging as an important nosocomial pathogen. In our study over a period of one year (January 2002- December 2002) at BPKIHS, a tertiary care hospital, from a total no. of 1089 isolates from pus/ aspirates of ICU, Burns, Orthopedic and other post operative wards, 146 isolates (13.4%) were Acinetobacter spp. Out of 146, 23 isolates of *Acinetobacter* spp. were sensitive to base line drugs. Remaining 123 strains of *Acinetobacter* spp. were resistant to Cefotaxime (99.2%), Ceftazidime (98.4%), Tobramicin (95.9%), Amikacin (96.7%), Netilmicin (89.43%), Ciprofloxacin (96.7%). Apart from these antibiotics none of the strains were sensitive to Piperacillin.

Introduction:
Acinetobacter spp. are ubiquitous, aerobic gram negative coccobacilli which are opportunistic pathogens with increasing relevance in nosocomial infections1. They cause a wide range of clinical complications such as septicemia, pneumonia, meningitis, wound infections and urinary tract infections (UTI) especially in immunocompromised patients1. Risk factors for acquisition of these organisms include prolonged hospital stay, serious underlying disease, intravascular and intravesical catheterization and treatment with broad-spectrum antibiotics2. Due to life threatening potential of such infections, empiric treatment with broad-spectrum antimicrobial agents is mandatory while awaiting organisms identification and in vitro susceptibility test results. However, increasing antimicrobial resistance in *Acinetobacter* spp. has effectively eliminated by many treatment alternatives, raising concerns about optimum therapeutic regimens3.

The purpose of the study was to determine the in vitro susceptibility of *Acinetobacter* isolates obtained from the indoor patients in BPKIHS, to various antimicrobial agents by disk diffusion method.

Materials and Methods:
Identifications:-
One hundred forty-six *Acinetobacter* isolates obtained from Blood, CSF, Pus, E.T.T and other body fluids from patients of ICU, Burns, Orthopedic and other post operative wards for a period of one year were taken.(Table.1).

Table 1:

Nature of sample	Nos. of Acinetobacter Isolates
Pus	62
E.T.T	47
Blood	19
CSF	05
Others	13

The strains were identified as being of the genus *Acinetobacter* on the basis of Bailey Scott's diagnostic Microbiology 9th edition (Table.2).

Table-2

Acinetobacter spp.	Growth at 42°C	Gelatin liquification	Urease	Citrate	Growth at 37oC	Acid prodn O/F glucose
A.calcoaceticus	-	-	+/-	+	+	+
A.baumanii	+	-	-	+	+	+
A.haemolyticus	-	+	-	+	+	+
A.jonsonii	-	-	-	+	-	-
A.junii	+	-	-	+	+	-
A.lwoffi	-	-	-	-	+	-

Preparation of inoculum and antimicrobial susceptibility testing:-

Fine similar looking colonies of the test organism were picked up with sterile loop and suspended into peptone water and incubated at 370C for 2-4 hours. The turbidity of the suspension was adjusted to McFarland's Nephalometer standard tube no. 0.5 (1.5x108CFU/ml).

Commercially prepared antibiotic discs (Hi-Media Ltd., India) of 6 mm. in diameter were used to determine the susceptibility pattern of *Acinetobacter* species in Mueller Hilton Agar (MHA) media. Kirby-Bouer's disk diffusion susceptibility testing (6 antimicrobial discs/plate) was performed for the following antimicrobial agents with their concentration given in parenthesis: Ampicillin (10ìg), Amikacin (30ìg), Cefazolin (30ìg), Cefotaxime (30ìg), Ceftazidime (30ìg), Ciprofloxacin (05ìg), Gentamicin (10ìg), Tobramicin (10ìg), Netilmicin (30ìg), Piperacillin (100ìg).

The plates were incubated at 370C for overnight and the results were determined.

For control, the organisms *Escherichia coli* (ATCC 25922 and *Pseudomonas aeruginosa* (ATCC 27853) were used.

Zone of inhibition (diameter of the circular inhibition zone including the antimicrobial discs) was measured by using Vernier Caliber and interpreted as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Results:

A total 146 isolates were identified as a genus *Acinetobacter.* First these isolates were subjected for the sensitivity against the base line drugs and only 23 out of 146 isolates were sensitive to them, and the remaining 123 base line resistant isolates subjected to the sensitivity against the 2nd line drugs.

Table-3

Group of Antibiotics	Antibiotics	Resistance (%)
3rd Gen.Cephalosporins	Cefotaxime	99.2
	Ceftazidime	98.4
Aminoglycosides	Gentamicin	96.6
	Tobramicin	95.9
	Amikacin	96.6
	Netilmicin	89.4
Quinilones	Ciprofloxacin	96.6
â-lactam	Piperacillin	100.0

The resistant pattern of these isolates are as follows (Table 3). 3rd generation Cephalosporins (98.4-99.2%), Aminoglycosides (90.0-97.0%), and Quinilones (97.0%). Apart from these none of the strains were found sensitive to Piperacillin.

Discussion:

Nosocomial bacterial pathogen show resistance pattern which may vary widely from time to time and place to place and within the same place over time. Only due to this, regular surveillance of nosocomial pathogen for resistograms is needed for every hospital in order to guide the appropriate selection of antimicrobials for empiric therapy. Monitoring the resistance of nosocomial pathogen could also be a primary pointer for the emergence of an outbreak. Detection of resistance in a particular pattern may suggest a currently occurring epidemic in hospitals, but antibiogram alone may not be sufficient to distinguish two strains that were responsible for outbreak.

A.baumannii which is generally multi drug resistant, is involved in several outbreaks more often than any other species of *Acinetobacter.*4,6,7 The incidence of nosocomial infection caused by *Acinetobacter* is rarely reported in Nepal, as compared to other south Asian countries.8,9 Antibiotic resistance is a major problem for patients infected with all *Acinetobacter* spp., especially those with *A. baumanni.i*4,5,10 This effect the appropriate antibiotic selection for treating such patient. In India also only few authentic data are available regarding *in vitro* susceptibility of clinical isolates of *Acinetobacter* spp.16,17

By the disc diffusion method it was clear that aminoglycosides were relatively more active than 3rd generation cephalosporins and â-lactam against the *Acinetobacter* spp. But quinilones (97.0% resistance) have shown almost the same results as compared to 3rd generation cephalosporins and â-lactam with aminoglycosides. Increasing resistance for cephalosporins was observed mainly in strains belonging to *A.baumannii.* The ranges of these antibiotics against *Acinetobacter* spp. are, 3rd generation Cephalosporins (98.4-99.2%), Aminoglycosides (90.0-97.0%), and Quinilones (97.0%). Now it seems clear from this data that all group of antibiotic has nearly the same antibiogram with >90.0% resistance against *Acinetobacter* spp. Ü and â-lactam Piperacillin was 100% resistant to *Acinetobacter* spp., suggesting that most of the first generation drugs were ineffective. Thus the agents which were used two decades earlier to treat *Acinetobacter* infections were now inactive against this bacterium and consequently these antibiotics are not useful in treating *Acinetobacter* infection.4,5,11

The isolates of *Acinetobacter* spp. showed maximum level of activity with Netilmicin whose susceptibility is 10.6%. Netilmicin was superior to other aminoglycosides especially Amikacin. Cefotaxime and Ceftazidime showed almost the similar pattern of resistance against the *Acinetobacter* spp. *i.e.*99.2% and 98.4% respectively. Only 3.0% isolates of *Acinetobacter* spp. were found sensitive to Ciprofloxacin. But Piperacillin (â-lactam) was 100.0% resistant to these isolates.

High level of resistance were noticed for Ceftazidime 98.4%, this is different from the results reported from Turkey and Greece, where in 67.5% and 96.0% was witnessed respectively for Ceftazidime.13,14 And was extreamly different with an Indian report according to which the resistance pattern is 37.0%18 for Ceftazidime against *Acinetobacter* spp. Amikacin showed 96.6% resistance, in contrast, Chang *et al.* reported higher susceptibility rates (74.5%) among *Acinetobacter* spp. strains for Amikacin.12 High percentage of strains belonging to *Acinetobacter* spp. were resistant to Ciprofloxacin (97.0%) by disc diffusion method. *Acinetobacter* strains were more resistant to quinolones when compared to other studies in Chile.12,15 While very similar to the results seen in Germany.

In summary, Strains of *Acinetobacter* spp. from patient in our Hospital were generally more resistant to quinolones, â-lactam antibiotics, first second and third generation Cephalosporins and aminoglycosides. However, dispite such resistance pattern, combination therepy could be the best choice for treating *Acinetobacter* infections in our hospitals.

References:

1. German Bou, Gonzalo *et al.* Characterization of a nosocomial Outbreak caused by a Multiresistant *A.baumannii* strains with a Carbapenem-hydrolysing enzyme: High level resistance in *A.baumannii* is not due solely to the presence of â-lactamases. *J Clin Microbiol* 2000;**38**:3299-3305.
2. Johannes G, Koeleman *et al*. Comparison of Amplified Ribosomal DNA Restriction analysis Random amplified polymorphic DNA analysis, And Amplified fragment length Polymorphism fingerprinting for identification of *Acinetobacter* genomic spp. and typing of *Acinetobacter baumannii. J Clin Microbiol* 1998;**36**:2522-29.
3. Marisa B Marques, Eneida S *et al.* Comparetive in vitro antimicrobial susceptibilities of nosocomail isolates of *Acinetobacter baumannii* and synergistic activities of nine antimicrobial combinations. *Antimicrobial.agent.chemther*.1997;**41**:881-85
4. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as Nosocomial Pathogen; Microbiological, Clinical, and epidemiological features. *Clin Microbiol Rev* 1996;**9**: 148-65.
5. Seifert H, Baginski R, Schulze A, Pulverer G. Antimicrobial susceptibility of *Acinetobacter* spp. *Antimicrob Agents Chemother* 1993;**37**: 750-53.
6. Siegman-Igra Y, Bar-Yosef S, Gorea A, Avram J. Nosocomial *Acinetobacter* meningitis secondary to invasive procedure: Report of 25 cases and review. *Clin Infect Dis* 1993;**17**:843-49.
7. Beck-Sague CM, Jarvis WR, Brook JH, Culver DH, Potts A, Gay E, Shotts DW, Hill B, Anderson RL, Weinstein MP. Epidemic bacteremia due to *Acinetobacter baumannii* in five intensive care units. *Am J Epidemiol* 1990; **132**: 723-33.
8. Kapila A, Gulati S, Goel V, Kumar L, Krishnan R, Kochupillai V. Outbreak of nosocomial *Acinetobacter baumannii* bacteremia in a high risk ward. *Med Oncol* 1998;**15**:270-74.
9. Iqbal Hussain M, Iqbal Kabir AK, Khan WA, Fuchs GJ. *Acinetobacter* bacteraemia in patients with diarrhoel disease. *Epidemiol Infect* 1998;**120**:139-42.
10. Bauernfeind A, Kljucar S, Jungwrith R. Overview of antibiotic resistance problems in *Acinetobacter* spp. In: Clinical importance and antibiotic resistance of *Acinetobacter* spp. Proceedings of a symposium held on 4-5 Nov.1996 Towner KJ, (ed) (Eilat,Israel). *J Med Microbiol* 1998;**46**: 726-28.
11. Traub WH, Spohr M. Antimicrobial drug susceptibility of clinical isolates of *Acinetobacter* spp. *Antimicrob Agents Chemother* 1989;**33**:1617-19.
12. Chang SC, Chen YC, Luh KT, Hsieh WC. In vitro activities of antimicrobial agents, alone and in combination, against *Acinetobacter baumannii* isolated from blood. *Diagn Microbiol Infect Dis* 1995;**23**:105-110.
13. Turkish Antimicrobial Resistance Study Group, Pfaller MA, Korten V, Jones RN, Doern GV. Multicenter evalution of the antimicrobial activity for seven broad spectrum â-lactams in Turkey using the E-test method. *Diagn Microbiol Infect Dis* 1999;**35**:65-73.
14. Sofianou DC, Constandinidis TC, Yannacou M, Anastasiou H, Sofianos E. Analysis of risk factors for ventilator-associated pneumonia in a multidisciplinary ICU. *Eur J Clin Microbiol Infect Dis* 2000; **19**:460-63.
15. Bello H, Gonzalez G, Dominguez M Zemelman R, Garcia A, Mella S. Activity of selected betalactams, Ciprofloxacin and Amikacin against different *Acinetobacter baumannii* Biotypes from chilean Hospitals. *Diagn Microbiol Infect Dis* 1997;**28**:183-86.
16. Patil JR, Chopade DA. Distribution and in vitro antimicrobial susceptibility of *Acinetobacter* spp. on the skin of healthy humans. *Natl Med J India* 2001;**14**:204-08.
17. Pandey A, Kapila A, Sood S, Goel V, Das B, Seth P. In vitro activities of ampicillin-sulbactum and amoxicillin- clavulanic acid against *Acinetobacter baumannii. Lin icrobiol* 1998;**36**:3415-16.
18. Prashanth K, Badrinath S. In Vitro susceptibility pattern of *Acinetobacter* spp. to commonly used Cephalosporins, Quinolones and Aminoglycosides. *J Med Microbiol* 2004;**22**:97-103.
19. German Bou, Gonzalo *et.al* Characterization of a nosocomial Outbreak caused by a Multiresistant *A.baumannii* strains with a Carbapenem-hydrolysing enzyme: High level resistance in *A.baumannii* is not due solely to the presence of â-lactamases. *J Clin. Microbiol.* 2000;**38**:3299-3305.
20. Johannes G, Koeleman *et.al* Comparison of Amplified Ribosomal DNA Restriction analysis Random amplified polymorphic DNA analysis, And Amplified fragment length Polymorphism fingerprinting for identification of *Acinetobacter* genomic spp. and typing of *Acinetobacter baumannii. J Clin. Microbiol.* 1998;**36**:2522-29.
21. Marisa B Marques, Eneida S, *et.al.* Comparetive in vitro antimicrobial susceptibilities of nosocomail isolates of *Acinetobacter baumannii* and synergistic activities of nine antimicrobial combinations. *Antimicrobial.agent.chemther*.1997;**41**:881-885
22. Bergogne-Berezin E, Towner KJ. *Acinetobacter* spp. as Nosocomial Pathogen ; Microbiological, Clinical, and epidemiological features. *Clin. Microbiol. Rev* 1996;**9**: 148-65.
23. Seifert H, Baginski R, Schulze A, Pulverer G. Antimicrobial susceptibility of *Acinetobacter* spp. *Antimicrob Agents Chemother* 1993;**37**: 750-53.
24. Siegman-Igra Y, Bar-Yosef S, Gorea A, Avram J. Nosocomial *Acinetobacter* meningitis secondary to invasive procedure: Report of 25 cases and review. *Clin Infect Dis* 1993;**17(5)**:843-49.
25. Beck-Sague CM, Jarvis WR, Brook JH, Culver DH, Potts A, Gay E, Shotts DW, Hill B, Anderson RL, Weinstein MP. Epidemic bacteremia due to *Acinetobacter baumannii* in five intensive care units. *Am J Epidemiol* 1990; **132(4)**: 723-33.
26. Kapila A, Gulati S, Goel V, Kumar L, Krishnan R, Kochupillai V. Outbreak of nosocomial *Acinetobacter baumannii* bacteremia in a high risk ward. *Med Oncol* 1998;**15(4)**:270-74.
27. Iqbal Hussain M, Iqbal Kabir AK, Khan WA, Fuchs GJ. *Acinetobacter* bacteraemia in patients with diarrhoel disease. *Epidemiol Infect* 1998;**120(2)**:139-42.
28. Bauernfeind A, Kljucar S, Jungwrith R. Overview of antibiotic resistance problems in *Acinetobacter* spp. In: Clinical importance and antibiotic resistance of *Acinetobacter* spp. Proceedings of a symposium held on 4-5 Nov.1996 Towner KJ, (ed) (Eilat,Israel). *J Med Microbiol* 1998;**46**: 726-28.
29. Traub WH, Spohr M. Antimicrobial drug susceptibility of clinical isolates of *Acinetobacter* spp. *Antimicrob Agents Chemother* 1989;**33**:1617-19.
30. Chang SC, Chen YC, Luh KT, Hsieh WC. In vitro activities of antimicrobial agents, alone and in combination, against *Acinetobacter baumannii* isolated from blood. *Diagn Microbiol Infect Dis* 1995;**23(3)**:105-110.
31. Turkish Antimicrobial Resistance Study Group, Pfaller MA,

Korten V, Jones RN, Doern GV. Multicenter evalution of the antimicrobial activity for seven broad spectrum â-lactams in Turkey using the E-test method. *Diagn Microbiol Infect Dis* 1999;**35(1)**:65-73.

32. Sofianou DC, Constandinidis TC, Yannacou M, Anastasiou H, Sofianos E. Analysis of risk factors for ventilator-associated pneumonia in a multidisciplinary ICU. *Eur J Clin Microbiol Infect Dis* 2000;**19**:460-63.
33. Bello H, Gonzalez G, Dominguez M Zemelman R, Garcia A, Mella S. Activity of selected betalactams, Ciprofloxacin and Amikacin against different *Acinetobacter baumannii* Biotypes from chilean Hospitals. *Diagn Microbiol Infect Dis* 1997;**28(4)**:183-186.
34. Patil JR, Chopade DA. Distribution and in vitro antimicrobial susceptibility of *Acinetobacter* spp. on the skin of healthy humans. *Natl Med J India* 2001;**14(4)**:204-08.
35. Pandey A, Kapila A, Sood S, Goel V, Das B, Seth P. In vitro activities of ampicillin-sulbactum and amoxicillin- clavulanic acid against *Acinetobacter baumannii*. *Lin icrobiol* 1998;**36**:3415-16.
36. Prashanth K, Badrinath S. In Vitro susceptibility pattern of *Acinetobacter* spp. to commonly used Cephalosporins, Quinolones and Aminoglycosides. *J Med Microbiol* 2004;**22(2)**:97-103.