ejpmr, 2015,2(6), 314-319

SJIF Impact Factor 2.026

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

PHENOTYPIC CHARACTERIZATION OF ACINETOBACTER FROM VARIOUS CLINICAL SAMPLES AND MOLECULAR STUDY ON IMIPENEM RESISTANT STRAINS IN A TERTIARY CARE HOSPITAL, ENATHUR, KANCHIPURAM.

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Article Received on 20/09/2015 **Article Revised on 13/10/09/2015** **Article Accepted on 03/11/2015**

ABSTRACT

Background: Acinetobacter is a Gram negative nosocomial pathogen, incapable of causing infection on its own, however causes colonization in susceptible patients especially with underlying pathology such as neutropenia or organ transplants or CAPD or catheter induced bacteruria. It is one of the causative agent of nosocomial pneumonia. A study is done on its causative role among various infections like UT infections, wound infections, respiratory tract infection, Blood stream infections, for a period of 1year duration. Antibiotic susceptibility Pattern studied and by using PCR technique imipenem *bla* OXA genes were studied. **Materials and methods:** Acinetobacter is identified by various Biochemical tests such as sugar fermaentation, indole test, methyl red, VP, Citrate utilization test, TSI, Urease test, mannitol motility. Further studied for Antibiotic susceptibility by using Muller – Hinton agar and Kirby bauer method and molecular characterization by PCR. **Results:** Among 1665 urine samples received over a period of 1 year duration 523 (31.41%) were culture positive, among culture positives commonest isolate was Escherichia coli 230(43.97%), citrobacter was least common being 6 out of 523 where as Acinetobacter were 16/523 (3.05%).Among pus samples 553 were total samples 409 were culture positive(73.96%),Staphylococcus aureus 173/409 (42.29%) was being the most commonest causative organism and Entercoccus was least common 4/409(0.997%), Acinetobacter were causative agents for 8/409(1.95%).Among sputum samples total nuber were 1313 and culure positive were 512 (38.99%). Acintobacter was isolated from 15 cases (nosocomial) 15/512(3%). Among various fluids there were 31 isolates among 137 sample (19/147) however, Acinetobacter was not isolated and among blood stream infections 3/386 cases(0.77%) were positive for acinetobacter. **Conclusions:** Among various samples Acinetobacter was causative agent for only few casaes of 1 – 3% however common among hospital acquired infections.2 strains were resistant to imipenam and 2 strains were moderaetly resistant to imipenam .The present study shows their greater incidence among women with age group (50-60). Imipenem resistant strains were studied for *bla*OXA genes.

KEYWORD: Acinetobacter, pathology, using PCR technique imipenem *bla* OXA genes.

INTRODUCTION

The genus Acinetobacter includes a range of pathogenic species among them common causative agents are *A.baumannii* group, *A. lwoffii*, *A. johnsonii*, *A. junii*, *A.hemolyticus* and other at least 25 genomospecies.[1] Acinetobacter infections are commonly associated with humidifiers, ventilators, catheters and other divices.[2] Among indwelling catheter associated infections after pseudomonas aeruginosa Acinetobacter is known to be commonest non-fermenter.25%of adults carry the organism on their skin. Until recently the organism is considered to be insignificant in urine and respiratory specimens. Increased carbepenem resistance & its causative role among nosocomial infections [3] made acinetobacter significant pathogen. They account for 1-3% of hospital acquired infections. The infections due to Acinetobacter include UT infection, endocarditis, Pneumonia, tracheobronchitis, septicemia, meningitis, cellulitis, conjunctivitis, corneal ulcer, endophthalmitis etc.[4]

Acinetobacter is resistant to 1st and 2nd generation cephalosporins, fluoroquinalones, variably sensitive to aminoglycosides and piperacillin Tazobactum combination. Many strains are resistant to carbepenems (CRAB) which are susceptible to colistins and tigecyclins.[5] The nosocomial infections due to Acinetobacter are common and the strains isolated among them are multidrug resistant that made them an important emerging pathogen known since long time.[6]
A study is made on the acinetobacter infections among various isolates in our tertiary care hospital. From various samples such as urine, pus, sputum, blood, fluids the incidence of acinetobacter infections were found to

be 0-3%.Among 42 isolated strains from different samples 24 were females and 18 were males. The infections were found to be common among nosocomial infections. Among all gram negative bacteria isolated from different samples only 4 strains of Acinetobcter isolated from nosocomial infections were resistant to imipenem.

MATERIALS AND METHODS

The study includes both IP&OP cases and they were categorized into nosocomial and routine cases**.**Those whose hospital stay of more than 48 hours in the medical and surgical wards of medical teaching institution were taken as nosocomial infections and the study was undertaken during October11, 2014 to September, 2015. The various specimens included were urine, pus, respiratory samples (sputum, endo-tracheal aspirate and bronchoalveolar lavage), blood and body fluids (pleural fluid, cerebrospinal fluid etc) and stool. Specimens were plated using appropriate culture media (Mac-Conkey agar, Blood agar, Chocolate agar in some cases using special media based on clinical diagnosis). Standard culture methods were used and the isolates were further identified by using appropriate biochemical tests.

The specific nosocomial infections were diagnosed as per the criteria laid by the Centre for Disease Prevention and Control. Antibiotic susceptibility was tested by the Kirby-Bauer disc diffusion method.Remaining cases were taken as routine cases. For those with positive culture reports, repeat cultures were made weekly, till discharge for an evidence of new infection. Those with the similar isolates with the same antibiogram at subsequent cultures were reported to have a single episode of infection. Isolation of more than two organisms from a sample was considered as an evidence of contamination and the repeat sample was collected. The antimicrobial sensitivity was tested to the following antibiotics as per the relevance: AC– Amoxyclavm,G– Gentamicin, Ak – Amikacin, IMP- Imipenem, PIT- Piperacillin+Tazobactam, CA – Cefotoxime, TCC – Ticarcillin and Clavalunic acid.

Kirby- Bauer disk diffusion method

Standardized inoculum was inoculated with the help of sterile cotton swab on the surface of the agarplate & the plate was allowed to dry for 3-5 minutes. Discs of antimicrobial agents were applied to the surface of the agar plate & incubated at 37°c. After18 hours, the results were recorded by comparing with standard ATCC stains.

Minimum Inhibitory Concentration

Muller Hinton agar with 2% NaCl and inoculums of10^4cfu/ml were used to detect MIC. Serial dilutionsof antimicrobial agents were prepared 1.5ml of each dilution was added to 13.5ml of melted Muller Hinton agar suspension & poured into plates. Many strains isolated from various infection such as urine, sputum, pus/ burns/ swab inoculated & incubated at 35°c. After 24 hours, the results were recorded.[8,9,10,11]

RT-PCR

The concise description of detection of imipenem resistance genes by polymerase chain reaction (PCR)

The DNA from an overnight culture on blood agar was extracted according to the manufacturer's instructions. 5 µl of the DNA extract and the final elution volume 100 µl was used for each PCR analysis. Uniplex PCR assays were used to detect the following β-lactamase genes: four carbapenem-hydrolysing oxacillinases (*bla*OXA-51, *bla*OXA-23, *bla*OXA-24 and *bla*OXA-58). The required primers were used for PCR amplification of the carbapenemase genes.

RESULTS

Table 1.

S.NO	Age&sex	sample	AK	G	AC	IMP	CA	TCC	PIT	Site ofcollction	SPECIES ISOLATED
1	41/F	uine	S	S	S	S	S	S	S	PO wound/ortho	A.bauminnii
2	3m/F	uine	S	S	S	S	S	R	S	Paediatric ICU	A.bauminnii
3	21/F	uine	S	S	S	S	R	S	S	post partumL.ward	Acinetobacter
4	51/M	uine	S	S	S	S	S	S	S	Medicine catheter /UTI	A.bauminnii
5	53/M	uine	S	S	R	R	R	R	R	ICU(nosocomial)	A.bauminnii
6	26/F	uine	S	S	S	S	S	S	S	post partumL.ward	Acinetobacter
7	57/F	uine	S	S	S	S	S	S	S	Medicine catheter /UTI	A.lwoffi
8	83/F	uine	S	S	S	MS	S	S	S	Medicine catheter /UTI	A.bauminii
9	58/F	uine	S	S	R	S	S	R	R	EMR	Acinetobacter
10	65/F	uine	S	S	MS	S	S	S	S	Medicine catheter /UTI	A.bauminnii
11	54/M	uine	S	R	R	S	R	R	R	Diabetic ward	A.bauminnii
12	7 m/M	uine	S	S	S	S	S	S	R	PO wound.I/Gynecology ward	A.bauminnii
13	27/F	uine	S	S	S	S	S	S	S	ICU	Acinetobacter
14	59/F	uine	S	S	S	S	S	S	S	post partumL.ward	A.lwoffi
15	43/F	uine	S	S	S	S	S	S	S	Medicine catheter /UTI	Acinetobacte
16	46/F	uine	S	S	S	S	S	S	S	PO wound.I/Gynecology ward	Acinetobacte
17	45/F	pus	R	R	R	S	R	R	R	Left leg abscess	A.bauminni
18	45/F	pus	S	R	R	MS	S	S	S	Wound site fasciotomy(abdomen)	A.bauminni

19	45/M	pus	MS	R	R	S	R	R	R	Wound site(surgical)	A.bauminni
20	52/F	pus	S	R	R	S	R	R	R	Wound site(surgical	A.bauminni
21	60/M	pus	S	S	R	S	S	S	S	Diabetic foot	A.bauminni
22	70/M	pus	R	R	S	S	R	R	R	Wound site(surgical)	A.lwoffi
23	33/M	pus	S	R	R	S	R	R	R	Wound site(surgical)	A.bauminni
24	61/F	pus	R	R	R	S	R	R	R	Wound site(surgical)	A.bauminni
25	60/M	sputum	R	R	R	R	S	S	S	Medicine(RTI)nosocomial	A.bauminni
26	65/F	sputum	S	S	S	S	S	S	S	TBCD ward	Acinetobacter
27	65/F	sputum	S	R	R	S	R	R	S	Medicine(RTI)nosocomial	A.bauminni
28	60/M	sputum	S	S	S	S	S	S	S	Medicine(RTI) OP	A.bauminni
29	46/F	sputum	R	R	R	MS	R	R	R	oral suction tube	A.bauminni
30	17/F	sputum	S	S	R	S	R	S	S	Medicine(RTI)nosocomial	A.bauminni
31	58/F	sputum	R	R	R	S	MS	S	R	Medicine(RTI)nosocomial	A.bauminni
32	54/M	sputum	S	S	S	S	S	S	S	Medicine(RTI)nosocomial	A.bauminni
33	55/F	sputum	S	S	S	S	S	S	S	Medicine(RTI) OP	A.bauminni
34	80/M	sputum	S	S	S	S	S	S	S	Medicine(RTI) OP	A.bauminni
35	42/M	sputum	R	R	R	S	R	R	R	Medicine(RTI)nosocomial	A.bauminni
36	80/M	sputum	S	S	S	S	S	S	S	Medicine(RTI) OP	A.bauminni
37	13/M	sputum	S	S	S	S	S	S	S	Medicine(RTI) OP	A.bauminni
38	75/F	sputum	S	S	S	S	S	S	S	Medicine(RTI) OP	A.lwoffi
39	64/M	sputum	S	S	S	S	S	S	S	Surgical ward	Acinetobacter
40	20days/F	blood	S	S	S	S	S	S	S	Paediatric ICU	A.bauminni
41	4 m/M	blood	S	S	S	S	S	S	S	Paediatric ICU	Acinetobacte
42	2 ½ m/M	blood	S	S	S	S	S	S	S	Paediatric ICU	A.bauminni

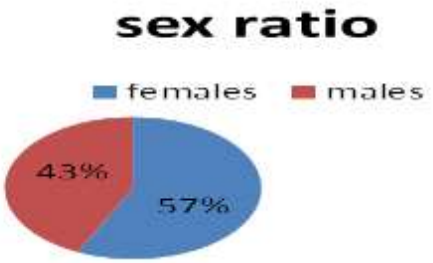

Figure 1

Out of 42 strains of Acinetobacter isolated from various infections. 33/42 (78.57%) of the strains were isolated from nososcomial infections. Among them 24 cases were of females and 18 cases were males. Acinetobacter isolated from pus (post operative wound infections) were showing multiple antibiotic resistance(MAR). Among them 4 strains were resistant to imipenem and they were found to be panresitant strains. They were further tested for second line antibiotics such as CAC (Cefotozidime +clavalunic acid) CPT (cefipime+tazobactum) CIT (ceftriaxone+tazobactum) SC (Sparfloxacin) Net (netilmycin) LF (levofloxacin) OF (ofloxacin).

Table 2

Age	No of Acinetobacter infections
0-10	5
11-20	2
21-30	3
31-40	1
41-50	8
51-60	13
61-70	6
71 and above	4
Total	42

The age distribution among 42 isolated was as follows Maximum number of cases were isolated among the age group between 51 – 60.minimum among the age groups between 31- 40.

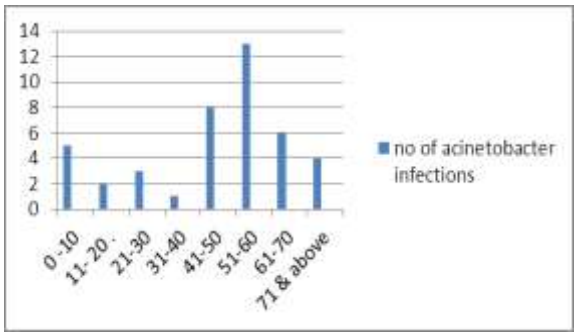

Figure 2

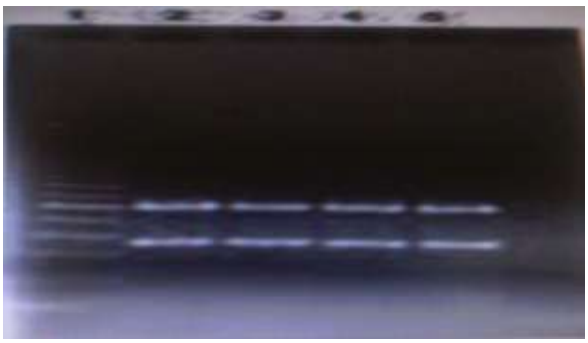

figure 3

***Bla*OXA genes detected in imipenem resistant Acinetobacter.4 stains.LANE 1 is control band,2,3,4,5 represent 4 imipenem resistant stains of Acinetobacter.**

Antibiotic sensitivity pattern of various Acinetobacter sps isolated from different samples.

Table3

Antibiotic	Number sensitive	Percentage of R
AK	34	19.04%
G	29	30.9%
AC	27	35.71%
IMP	38	9.5%
CA	26	38.05%
TCC	28	33.33%
PIT	29	30.9%

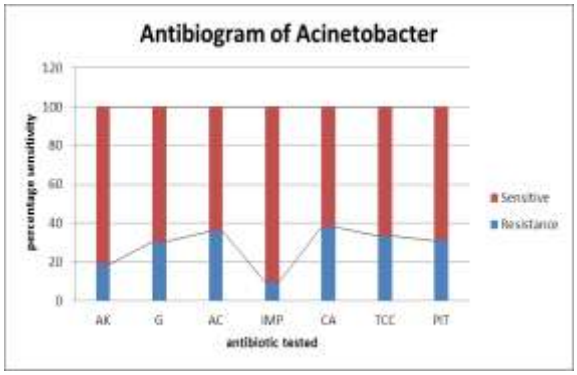

Figure 4

Antibiotic sensitivity by Kirby- Bauer technique showed maximum percentage of resistance to cefotoxime (38.05%) with minimum resistance to Imipenem(9.5%)

Table

The Table showing number of culture positive samples,among them various bacterial strains isolated.

S.NO	E.Coli	Klebsiella	CONS	Pseudomonas	Acinetobacter	Proteus	Staph .aureus	Strepto pyogenes	Entero cocci
URINE(523/1665)	240	70	99	25	16	20	17	8	17
PUS(477/553) (68dual isolates)	75	52	67	62	8	33	175	nil	4
SPUTUM(512/1313)	31	71	22	51	15	2	42	208	26
BLOOD (45/386)	11	2	15	8	3	1	3	0	2
FLUIDS	2	6	2	1	NIL	1	2	4	1

Citrobacter	Candida
2	10
1	nil
nil	22
nil	nil

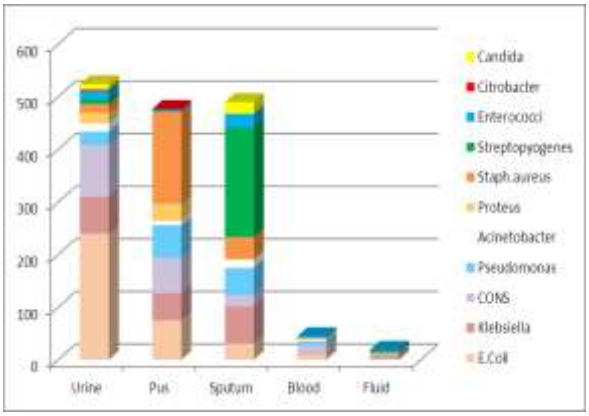

FIGURE 5 Culture positives among various clinical specimens

Among the different isolates from various samples between October 2014 to September 2015, the infections due to Acinetobacter range between 0 -3%, Among urine samples Acinetobacter were 16/523 (3.05%), among pus samples Acinetobacter were 8/409 (1.95%)Among sputum samples there were 15 cases /512(3%), among blood stream infections 3/386 cases(0.77%) were positive, among fluids there were no cases due to Acinetobacter. Out of total 1665 urine samples received in the laboratory 523 cases were culture positive .The majority of infections were due to Escherichia coli. 523/1665(31.41%).Among 553 pus samples 477 cases were culture positive (477/553 =86.25%) there were dual isolates for 68 cases.Among sputum samples out of 1313 total samples 512 samples were culture positive(512/1665 = 30.75%).Among fluids 19 cases were culture positive out of 147 cases.(12.82%).Among blood stream infections 45 cases were culture positive out of 386 caes. 45/386 (11.65%) Among 42 strains isolated from various infections Acinnetobacter bauminnii was isolated from 30 cases (71.42%) others 12 cases.

Table 5. comparison between the culture positives and number of Acinetobacter sps isolated.

Specimen	Total culture positives	Number of Acinetobacter isolated
Unine	523	16(3.05%)
Pus	477	8(1.95%)
Sputum	512	15(3%)
Blood	45	3(0.77%)
Fluids	19	Nil(0%)

DISCUSSION

Acinetobacter sps though responsible for o-3% infections among routine cases, their multidrug resistance is becoming much concern recently and there are cases

where not even a single drug sensitivity was detected by invitro method. The infections caused by these strains often becoming a significant threat in recent years.[7] Acinetobacter is inheritantly resistant to multiple antibiotics such as cephalosporins by producing Amp C cephalosporinases, Aminoglycosides by producing Aminoglycoside modifying enzymes, Quinalones by producing DNA gyrase enzyme.[13] It has become emerging pathogen among nosocomial infections. The organism is Gram negative, non-motile(Greek word akinetos means non motile), non – fermenter.[4]

In our present study 42 strains were isolated from total cases of 1576 culture positive cases (2.66%), however the majority of cases were isolated from nosocomial infections. This statastics are in correlation with many studies.[7]

The antibiotic sensivity pattern as detected by Kirby-Bauer method showed AK(19.04%), G(30.9%)AC(35.71%), IMP(9.5%)CA(38.05%), TCC(33.33%), PIT(30.9%). Cefotoxime resistance was found to be highest(38.08%). Its sensivity to cefotoxime may be attributable to loss of R mutational gene.[14] In Asia and the Middleeast,percentage of resistance is about 40% for Ceftazidime, 35% for Amikacin and 45% for Ciprofloxacin.

The multidrug resistant strains in our study was 12/42(28.57%). The study by Bhattacharya et al showed 29% pan resistant strains.[15,16]

In present study Imipenem resistance was found to be 9.5%.this is nearly correlating another study by Bhattacharya et al.,IN one study from USA imipenem resistance was found tobe 23.1%.[17]

Among 42 strains 24 cases were females and 18 were males and the commonest age group from which they were isolated were among 51-60 that suggests the infections were common among old age group with waning immunity. Many studies are showing males are affected commonly rather than females. In our study Acinetobacter was isolated most commonly from females.

In present study there were 4 strains of imipenem resistant strains that were further studied by molecular method. Interestingly all gram negative bacilli were sensitive to imipenem except for 4 strains of Acinetobacter. Only a few centres like I.M.S., B.H.U., Varanasi[18,19] and A.I.I.M.S., New Delhi, have mentioned imipenem resistance in *Acinetobacter* spp. to be about 6.4% and 22.16% respectively in their studies. Our study is correlating with their studies. Among Gram positive bacteria only coagulase negative staphylococci were found to be resistant to vancomycin 8 out of 67 isolated strains from pus samples. Were showing pan resistance.(wound infections)**.** Thus, Periodic surveillance using molecular typing of isolates from patients is needed for early detection of an epidemic strain, that consequently serves as an effective control measure.[20]

Emperical treatment for Acinetobacter infections in emergency cases i.e., to formulate antibiotic policy for Acinetobacter is challenging and it differs for given geographical area.[21] Antibiotic sensitivity testing provides a useful guide and institutional data monitoring as well as retrospective analysis are of great use in this regard.[22]

The overall isolation of the pathogens and their antibiotic policy was found to be normal except that coagulase negative Staphylococcus is emerging as pathogen among all samples in our geographical area.

CONCLUSIONS

Inadvertant use of antibiotics is responsible for increased resistance among the nosocomial infections.By differentiating colonization from infections and judicious use of antibiotics i.e., treating the infections only in clinically confirmed Acinetobacter infections can limit this evolving phenomena especially among nosocomial infections.

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