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ACINETOBACTER SPECIES;

PREVALENCE AND SENSITIVITY PATTERN OF ACINETOBACTER SPECIES AMONG CLINICAL ISOLATES OF TERTIARY CARE HOSPITAL

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ABSTRACT… Introduction: The genetic competencies of bacteria and the resistance have been impeding the usefulness of antibiotic therapy. There has been an alarming increase in the infections caused by Acinetobacter spp. especially the multidrug resistance pattern has narrowed the therapeutic ranges. **Objectives:** To determine the prevalence and antibiotic sensitivity pattern of Acinetobacter spp., among clinical specimens of tertiary care hospital. **Study Design:** Descriptive study. **Place & Duration of Study:** Pathology Department, PGMI, from January 2015 to December 2015. **Materials & Methods:** Total 8465 clinical specimens were inoculated. Acinetobacter spp. was identified and isolated by the preliminary microbiological and biochemical tests. Antimicrobial susceptibility testing was implemented by modified Kirby-Bauer disk diffusion method as per CLSI guidelines (2015). **Results:** Acinetobacter spp. isolated in 234 (7.29%) clinical specimens among 3208 (37.89%) culture positive isolates. Out of total 234 Acinetobacter spp. isolates 144 (61.54%) were recovered from male patients and 90 (38.46%) from female patients. the frequency of Acinetobacter spp. isolates was seen highest in CSF (23.07%) and lowest in HVS (5.52%) specimens. Maximum samples were recovered from surgical wards 85 (36.32%), while from pediatrics department only 20 (8.54%) samples. Only, 140 (59.82%) isolates were sensitive to tigecycline; while 216 isolates were (92.30%) resistant to salbactam. **Conclusion:** The progressively increasing threat of Acinetobacter resistant infections can be minimized by judicial use of antibiotics, and implementation of strict infection control policy in health care settings.

Key words: Acinetobacter spp. (species); ESKAPE Pathogens (E. Faecium, S. Aureus, K. Pneumoniae, A. Baumannii, P. Aeruginosa and Enterobacter spp.); CLSI (Clinical and Laboratory Standard Institute); MDR (Multidrug Resistance).

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INTRODUCTION

The genetic competencies of bacteria and careless use of antibiotics have ensued in the well-known expansion of resistance, impeding the usefulness of antibiotic therapy. Resistance to single antibiotic has further progressed into multidrug resistance which favorably shields bacterial pathogens against several frequently used therapeutic agents.[1] Acinetobacter baumannii belongs to one of the six ESKAPE pathogens, whose infections have recently been recognized as a grave emerging problem.[2] The major risk factors for acquisition of infection with Acinetobcter spp., in health care setting are: prolong hospitalization, ICUs (ventilator associated pneumonia), surgical procedures, blood stream infections (BSI), device-associated infections (urinary and intravascular catheters), and immunosuppression (renal failure, chronic lung disease, diabetes, terminal illnesses like cancer).[3] A number of Acinetobcter baumannii resistance mechanisms are known including acquirement of β-lactamases causing degradation of drugs, up-regulation of multidrug efflux pumps, modification of aminoglycosides, permeability defects in cell wall channels (porins), and alteration of target sites.[4] Increasing multidrug resistance pattern by Acinetobacter spp.; has narrowed the choice of treatment by antibiotics. The proper microbiological and biochemical identification; optimal selection of antibiotics for susceptibility testing according to CLSI guidelines along with vigilant employment of antibiotics; can help in abating the morbidity and mortality caused by Acinetobacter infections.

This objective of this study was to determine the prevalence; and antibiotic sensitivity pattern of Acinetobacter spp.; among clinical isolates of tertiary care hospital.

MATERIAL AND METHODS

Sample Collection and Processing

This descriptive study was conducted in Pathology Department of PGMI, Lahore; during the period from January 2015 to December 2015. Various clinical specimens e.g., blood, CSF, pus/wound swabs, HVS, CVP tip, tracheal secretion, fluid and urine; were received from patients admitted in different clinical wards of Lahore General Hospital (LGH). The specimens were processed according to standard operating procedures in microbiology laboratory of Pathology department, PGMI, Lahore.

Culture and Identification

All the samples were primarily inoculated on blood agar and MacConkey agar; while Cystine Lactose Electrolyte Deficient (CLED) medium was used for inoculation of urine samples. The plates were incubated aerobically at 37°C for 24 hours. The culture plates were examined for bacterial growth and colony morphology was noted using hand lens. Organisms were identified by standard microbiological and biochemical methods; including Gram staining, hanging drop, catalase and oxidase test. On gram staining, Acinetobacter strains were identified as gram negative bacilli or coccobacilli, non-motile, oxidase and catalase positive. Each strain was inoculated on triple sugar iron (TSI) to see the sugar fermentation reactions

Susceptibility Testing

Antibiotic susceptibility of the Acinetobacter isolates was determined by employing modified Kirby-Bauer disc diffusion method according to CLSI guidelines (2015). For each strain of Acinetobacter, a bacterial suspension adjusted to 0.5 McFarland turbidity standards was prepared and inoculated on Mueller Hinton agar (MHA). Antibiotic discs of Salbactam, Imipenem (10ug), Piperacillin-Tazobactam (100/10ug), Tigecycline (15ug), Cefotaxime (30ug), Ceftriaxone (30ug) – Ceftazidime (30ug), Cefepime (30ug), Aztreonam, Ciprofloxacin (5ug), Gentamicin (10ug), Amikacin (30ug), Doxycycline, and Tetracycline (30ug) were applied; and the plates were incubated at 35˚C for 24 hours. Acinetobacter baumannii (ATCC) 19606 was used as the quality control strain.

Statistical Analysis

Data will be entered and analyzed using SPSS Version 20.0 (Statistical Package for Social Sciences). Qualitative variable i.e., specimen isolation according to gender, type of specimen from different clinical wards and antimicrobial susceptibility were presented as frequencies and percentages. P values <0.05 are considered statistically significant.

RESULTS

During the study period from January 2015 to December 2015; a total of 8465 different clinical samples were received from Lahore General Hospital. Out of all the samples processed, 3208 (37.89%) were growth positive after culture. The overall frequency of Acinetobacter spp. isolates was 234 (7.29%) in 12 months, as shown in Table-I. This Table also reveals the distribution of Acinetobacter spp. isolates from different clinical specimens (n=234). According to different specimens, the frequency of Acinetobacter spp. isolates was highest being isolated from CSF (23.07%), followed by CVP tip (18,68%), Fluids (8.49%), Tracheal secretion (7.72%), Blood (6.39%), Urine (6.34%), Pus/Wound Swab (5.93%), and HVS (5.52%). Statistically the difference was significant (p< 0.05) among percentage of Acinetobacter spp. isolates from different clinical specimens.

Specimen	Positive growth	Acinetobacter spp.	
		No.	%age
Pus/ Wound Swab	961	57	5.93
Blood	594	38	6.39
Urine	599	38	6.34
CSF	130	30	23.07
HVS	507	28	5.52
CVP Tip	91	17	18.68
Tracheal Secretion	220	17	7.72
Fluid	106	9	8.49
Total	3208	234	7.29

Table-I. Distribution of Acinetobacter spp. isolated from different clinical specimen (n=234)

Figure-1 of our study shows; Breakup of Acinetobacter spp. isolates, from patients according to gender (n=234). Out of 234 isolates of Acinetobacter spp., 144 (61.54%) were recovered from male patients and 90 (38.46%) from female patients. It shows that frequency was more in males as compared to females. However, the difference was statistically non-significant (p 0.05).

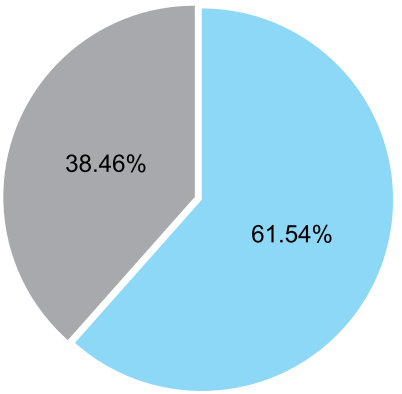

Figure-1. Breakup of Acinetobacter spp. isolates from patients according to gender (n=234)

Table-II shows the frequency of Acinetobacter spp. isolates from various clinical wards (n=234). According to this, maximum samples were recovered from surgical wards 85 (36.32%), followed by medicine 47 (20.08%), orthopedics 33 (14.10%), gynecology 28 (11.96%), neurology 21 (8.97%) and from pediatrics department 20 (8.54%). Statistically, the difference was non-significant (p > 0.05).

Ward	Frequency	Percentage (%)
Surgery	85	36.32
Medicine	47	20.08
Ortho	33	14.10
Gynae	28	11.96
Neuro	21	8.97
Paeds	20	8.54

Table-II. Frequency of Acinetobacter spp. isolated from various clinical departments (n=234)

Sensitivity pattern of Acinetobacter spp. isolates to various antibiotics (n=234) is described in Table-III of our study. It shows that 216 isolates were (92.30%) resistant to salbactam, followed by 210 (89.74%) to ceftriaxone, 190 (81.19%) to cefotaxime, 172 (73.50%) and 162 (69.23%) to amikacin and gentamicin respectively; 164 (70.08%) to ciprofloxacin, 160 (68.37%) and 156 (66.66%) to aztreonam and tetracyclin respectively. However, 147 (62.82%), 140 (59.82%) and 129 (55.125) isolates were sensitive to doxycycline, tigecycline, and peracillin-tazobactam, respectively.

No.	Antibiotics	Sensitive (%)	Resistant (%)
1	Salbactam (SAM)	18 (7.69)	216 (92.30)
2	Imipenem (IPM)	90 (38.46)	144 (61.53)
3	Piperacillin-Tazobactam (TZP)	129 (55.12)	105 (44.87)
4	Tigecycline (TGC)	140 (59.82)	94 (40.17)
5	Cefotaxime (CTX)	44 (18.80)	190 (81.19)
6	Ceftriaxone – Ceftazidime(CRO/CAZ)	24 (10.25)	210 (89.74)
7	Cefepime (FEP)	80 (34.18)	154 (65.81)
8	Aztreonam (ATM)	74 (31.62)	160 (68.37)
9	Ciprofloxacin (CIP)	70 (29.91)	164 (70.08)
10	Gentamicin (CN)	72 (30.76)	162 (69.23)
11	Amikacin (AK)	62 (26.49)	172 (73.50)
12	Doxycycline (DO)	147 (62.82)	87 (37.17)
13	Tetracyclin (TCN)	78 (33.33)	156 (66.66)

Table-III. Sensitivity pattern of Acinetobacter spp. to different antibiotics (n=234)

DISCUSSION

The emergence of pan-resistance in bacterial pathogens has become a great threat to human health.[5] Among these, the multi-drug resistant Acinetobacter strains are important cause of nosocomial infections that are difficult to control and treat globally including in Pakistan.[6]

In the present study the prevalence of Acinetobacter spp. was found to be higher which is inconsistent with other studies conducted in Pakistan.[5,7] The higher isolation rates of Acinetobacter spp. was from the CSF in our study. A study from Turkey has documented Acinetobacter spp. as the leading cause of Gram-negative postneurosurgical meningitis.[8] In a review done by Kim et al[9], in 2009, describe that Acinetobacter is an increasingly important pathogen associated with postneurosurgical meningitis. A study conducted in Taiwan, Acinetobacter has been ranked the fifth most common genus to be associated with nosocomial meningitis.[10]

The resistance patterns of A. baumannii towards various antimicrobial agents were determined by disc diffusion method. A high percentage of antibiotic resistance of Acinetobacter spp. were detected for Sulbactam, Imipenem, Piperacillin-Tazobactam, Tigecycline, Cefotaxime, Ceftriaxone–Ceftazidime, Cefepime, Aztreonam, Ciprofloxacin, Amikacin, Doxycyclin and Tetracyclin which is corroborated with findings of previous reports in different hospitals of Iran, Turkey and Italy hospital.[11,12]

Our study showed highest percentage of resistance of Acinobacter Spp. to Salbactum. A study conducted by Begum et al[13] in 2013, A. baumannii exhibited the highest resistance 100% against β-lactam inhibitors. However, a large number of studies have shown a high percentage of sensitivity and effectiveness of Salbactam based therapies for the treatment of Acinobacter strains.[14]

High frequency of carbapenem resistance (61.53 %) was observed in our study. Our results are in concordance with Hussein et al[15] in 2013 in which (58.26%) isolates showed resistance to carbapenem. Similarly, a study done by Anwar et al[16] in children hospital Lahore found that 61% isolates of Acinetobacter were resistant to carbapenem.

It was observed in this study that 44.87 % isolates were resistant while 55.12% were sensitive to it. Henwood et al[17] in 2002 also showed resistant strains of Acinetobacter to Piperacillin-tazobactam.

Tigecycline always remains a good treatment options for the management of many cases of infections caused by multidrug resistant strains of Acinetobacter.[7] However, our study showed 40% resistance to tigecycline. Similarly, the emergence of Acinobacter strains with resistance against tigecycline is being reported in various studies.[18] Regarding Monobactams, our study revealed that A. baumannii showed resistance in 68.37% isolates.

High resistance of A. baumannii to third and fourth generation cephalosporins was reported in our study. Previously other researchers also showed high percentage of resistance among Acinetobacter Spp. against third and fourth generation cephalosporins.[19] [29, 30]. Fluoroquinolone (Ciprofloxacin) resistant strains in our study were 70.08 % while sensitive strains were only 29.91 %. Our results are in concordance with the study conducted by Shamim et al[20] in which 88.75% strains were resistant to ciprofloxacin.

CONCLUSION

The present study revealed the presence of high prevalence of multiantibiotic resistance isolates of Acinetobacter spp. in a tertiary care hospital of Lahore. The progressively increasing resistance patterns of Acinetobacter infections pose a warning as therapeutic stalemate. It is recommended that implementation of strict infection control policy in every health care setting which includes: precision of sterilization & disinfection techniques, hand hygiene, application of universal safety precautions, and regular hospital surveillance; as well as optimizing judicial use of antibiotics; can help to minimize this challenge.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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1	Aneela Khawaja	Entire research work, Literature review, Methodology, analysis and write up.	
2	Faiqa Arshad	Sample collection and processing, help in write up.	
3	Sadaf Munir	Sample collection and processing.