

# Frequency and Antimicrobial Susceptibility Pattern of Multidrug Resistant *Acinetobacter* Species in Sharif Medical City Hospital, Lahore

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### ABSTRACT

**Objective:** To determine the frequency and antimicrobial susceptibility pattern of multidrug resistant *Acinetobacter* species in different clinical specimens of Sharif Medical City Hospital, Lahore.

**Methodology:** It was a cross-sectional study conducted in the Department of Pathology, Sharif Medical City Hospital, Lahore over a period of one and a half year (from May 2017 to October 2018). The study was approved by the ethical committee of the institution. A total of 2490 clinical specimens were received in the Pathology laboratory for culture and sensitivity from different departments of the hospital and were processed by conventional bacteriological methods for isolation and identification. The antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standard Institute (CLSI). The Statistical Package for Social Sciences (SPSS) version 25 was used for data entry and analysis.

**Results:** Out of the 2490 culture specimens, 51(2%) positive cultures for *Acinetobacter* species were obtained. Most of the patients with the positive culture were between 19 to 50 years of age. The various clinical specimens from which *Acinetobacter* species were isolated were 34 pus/wound swabs followed by 8 blood, 3 urine, 3 tips (2 center venous catheters, 1 endotracheal tube), 2 sputum and 1 body fluid. The majority of the specimens were received from various outpatient departments, ICU and nursery. Thirty one percent strains were sensitive and 6% were intermediate to cefoperazone-sulbactam. Most of the isolates were resistant to other antimicrobials. Five (9.8%) strains of *Acinetobacter* species were resistant to all of the antimicrobials.

**Conclusion:** The prevalence of *Acinetobacter* species is low in different clinical specimens for culture and sensitivity in Sharif Medical City Hospital, Lahore. Thirty one percent strains were sensitive and 6% were intermediate to cefoperazone-sulbactam. All the strains of *Acinetobacter* species were multidrug resistant. Five (9.8%) strains of *Acinetobacter* species were resistant to all of the antimicrobials. However, the emergence of infections by multidrug resistant *Acinetobacter* species is an alarming sign in the hospital setting.

**Keywords:** : Nosocomial pathogen. *Acinetobacter* species. Multidrug resistance.

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### INTRODUCTION

Hospital-acquired or nosocomial infections are a major challenge to patient safety. They are the sixth leading cause of death in the United States. A total of 1.7 million hospital-acquired infections occur and approximately 99,000 deaths are associated with hospital-acquired infections. Gram negative bacteria account for greater than 30% of hospital-acquired infections.<sup>1</sup> Nosocomial pathogens and multidrug resistance are a potential threat to humans worldwide.<sup>2,3</sup> Bacterial strains that are resistant to  $\geq 4$  classes of antimicrobials are called multidrug resistant.<sup>4</sup> Multidrug resistance contribute not only to major therapeutic stress but also cause significant morbidity and mortality nowadays. *Acinetobacter* species is an important cause of nosocomial infections worldwide. It is difficult to control and treat the infections caused by *Acinetobacter* due to its high resistance to antimicrobials.<sup>2,3</sup>

*Acinetobacter* species causes nosocomial infections mainly in patients who are immunodeficient. Members

of the genus *Acinetobacter*, particularly *Acinetobacter baumannii*, are associated with multiple infections including nosocomial pneumonia, skin & soft tissue infections, meningitis, local wound infections, bacteremia, sepsis and urinary tract infections.<sup>4,5</sup> The transmission of infections caused by *Acinetobacter* is attributed to its ability to withstand a wide range of temperature and pH conditions which favours its persistence in the hospital environment.<sup>6</sup>

*Acinetobacter* species are gram negative coccobacilli which belong to the family Moraxellaceae. These are aerobic, encapsulated, non-motile, non-spore forming that do not ferment glucose. They test positive for citrate and catalase but negative for indole and oxidase.<sup>7-9</sup> They are widely distributed in nature as saprophytes or commensals and act as opportunistic pathogens for humans.<sup>7</sup> The organisms are ubiquitous in nature particularly in soil & water and in the hospital environment. They may be isolated from instruments such as ventilator machine, humidifiers, mattresses and other equipment as well as from the skin of healthcare workers.<sup>10</sup> Outbreaks caused by *Acinetobacter* infections are often associated with the spread of distinctive strains and have been associated with contamination of medical care and surgical equipment such as respiratory therapy equipment, intravascular access devices, bedding materials and also linked with the transmission through the hands of hospital

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Received: November 2, 2018; Accepted: December 12, 2018

personnel. In the hospital settings, the persistence of endemic *Acinetobacter baumannii* strains seems to be associated with their widespread resistance to the major antimicrobial agents as well as their ability to survive long term on inorganic surfaces of the patient's immediate environment.<sup>4</sup>

*Acinetobacter* species especially *Acinetobacter baumannii* is prevailing worldwide as the leading nosocomial pathogen.<sup>11,12</sup> In critically ill patients, it is responsible for higher mortality rates.<sup>13</sup> Several cases have been reported from hospitals in UAE, Bahrain, Saudi Arabia, Palestine and Lebanon of multidrug resistant *Acinetobacter baumannii*.<sup>8</sup>

Globally multidrug resistance (MDR) has become a major health issue. It has been estimated that it will be the leading cause of a large proportion of deaths by the year 2050. Gram negative bacteria have a greater tendency for multidrug resistance and there is a shortage of drugs unfortunately that can provide effective treatment.<sup>14</sup> The precise mechanism of MDR is the unnecessary use of different antibiotics which besides causing high treatment expenditures also leads to the emergence of multidrug resistant bacterial strains.<sup>15</sup> Antimicrobial resistance has become extremely problematic for Asian countries. It has been estimated that more than 70% of antibiotic resistant cases occur in the Asia-Pacific region of the world.<sup>16</sup>

Multidrug resistant bacterial strains pose a challenge for the treatment and have evolved worldwide as a major cause of morbidity and mortality. Unfortunately, proper surveillance and documentation of such pathogens are lacking especially in developing countries. This study is planned to determine the prevalence of *Acinetobacter* species in different clinical specimens for culture and sensitivity in Sharif Medical City Hospital (SMCH) and its antimicrobial susceptibility pattern. Multidrug resistant *Acinetobacter* species are a major pathogen involved in hospital-acquired infections. This study will help us to decrease the frequency and transmission of infections caused by *Acinetobacter* species through strict implementation and adherence to infection control measures.

### METHODOLOGY

This was a cross-sectional study conducted over a period of one and a half year from April 2017 to October 2018 in the Department of Pathology, Sharif Medical City Hospital, Lahore. The study was approved by the ethical committee of the institution. A total of 2490 clinical specimens (blood, pus/wound swabs, urine, sputum, central venous pressure catheters, tips, body fluids) were received from different departments of the Hospital including intensive care units (ICU), nursery, medicine, surgery,

neurosurgery, obstetrics & gynaecology, pediatrics and various outpatient departments (OPDs). All the specimens except blood and urine were inoculated on blood, chocolate and MacConkey agar. For urine samples, cysteine lactose electrolyte deficient (CLED) agar was used. Blood samples were inoculated on blood and MacConkey agar. The inoculated culture media plates were placed in an incubator at 35-37°C for 24-48 hours. Presumptive identification of isolates was made by conventional bacteriological methods including colony morphology, gram staining and biochemical tests. The antibiotic susceptibility testing was determined by Kirby-Bauer disc diffusion method. The standard suspension of test organisms was made using 0.5% McFarland and they were inoculated on Mueller-Hinton agar plates. The antibiotic discs were placed on the plates, incubated for 24 hours and antibiotic zones were interpreted as per Clinical and Laboratory Standards Institute (CLSI) and Eucast guidelines.<sup>17,18</sup>

The following commercially available antimicrobial discs (Oxoid, Basingstoke, UK) with strengths recommended by CLSI were used: cefoperazone-sulbactam (SCF), meropenem (MEM), imipenem (IPM), ciprofloxacin (CIP), ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP), cefixime (CFM), tazocin (TZP), tetracycline (TE), gentamycin (CN), amikacin (AK) and trimethoprim-sulfamethoxazole (SXT). Bacterial strains that are resistant to  $\geq 4$  classes of antimicrobials are called multidrug resistant.<sup>4</sup> The zone diameter of cefoperazone ( $\geq 21$ mm) is used as reference for cefoperazone-sulbactam because the zone diameter of cefoperazone-sulbactam is not mentioned in CLSI and Eucast. The zone diameter of tigecycline ( $\geq 18$  mm) for Enterobacteraceae as mentioned in Eucast is used as reference for reporting tigecycline sensitivity in *Acinetobacter*.<sup>17,18</sup>

### STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) version 25 was used for data entry and analysis. The number of positive cultures, patient age, gender, relevant departments and different clinical specimens for culture & sensitivity and sensitivity to various antibiotics were expressed as frequency and percentage. A p-value of  $\leq 0.05$  was considered statistically significant.

### RESULTS

A total of 2490 clinical specimens were received in the Pathology Laboratory, out of which 51 positive cultures for *Acinetobacter* species were obtained. The prevalence of *Acinetobacter* species in clinical specimens was only 2%.

Among the positive cultures, 30(59%) were of females while 21(41%) were of males. Only 7(14%) patients were below 1 year of age, 5(10%) patients were between 1 to 18 years, 24(47%) patients were between 19 to 50 years and 15(29%) patients were above 50 years.

The various clinical specimens from which *Acinetobacter* species were isolated were 34(66.7%) pus/wound swabs followed by 8(15.7%) blood, 3(5.9%) urine, 3(5.9%) tips (2 center venous catheters, 1 endotracheal tube), 2(3.9%) sputum and 1(1.9%) cerebrospinal fluid (CSF). These results are shown in figure 1.

The distribution among the specimens received from different departments include ICU (8%), nursery (14%), medicine (2%), surgery (6%), neurosurgery (2%), obstetrics & gynaecology (2%), pediatrics (2%) and various outpatient departments (64%) as shown in figure 2.

The isolated *Acinetobacter* strains showed best zones of inhibition to cefoperazone-sulbactam. Thirty one percent strains were sensitive and 6% were

intermediate to cefoperazone-sulbactam. Six percent of strains were sensitive to tetracycline. Only 2% of strains were sensitive to ceftazidime, tazocin, trimethoprim-sulfamethoxazole and aminoglycosides (gentamycin, amikacin). Ninety one and 94% strains were resistant to imipenem & meropenem and ciprofloxacin respectively. All of the strains were resistant to cephalosporins (cefotaxime, ceftriaxone, cefepime and cefixime).

Tigecycline is a newer drug in glycolcycline class of antibiotics which is mainly used to treat skin and soft tissue infections. Tigecycline was applied in pus/wound and catheter tips samples. Out of the 37 positive samples of pus/wound and catheter tips, 14(37.8%) were sensitive and 23(62.2%) were resistant to tigecycline.

In our study, all the strains of *Acinetobacter* species were multidrug resistant i.e. they were resistant to  $\geq 4$  classes of antimicrobial drugs. Five (9.8%) strains of *Acinetobacter* species were resistant to all of the antimicrobials.

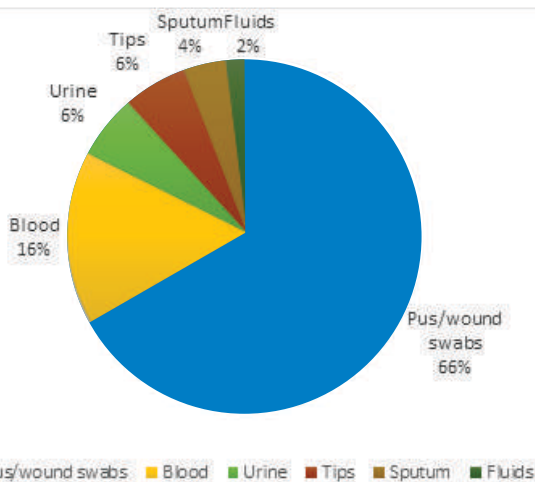


Figure 1: Distribution of *Acinetobacter* Species from Different Clinical Specimens

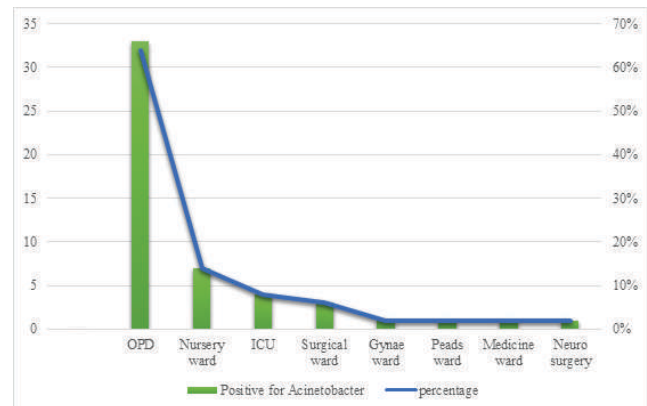


Figure 2: Isolation of *Acinetobacter* Species from Various Hospital Departments

Clinical Specimens

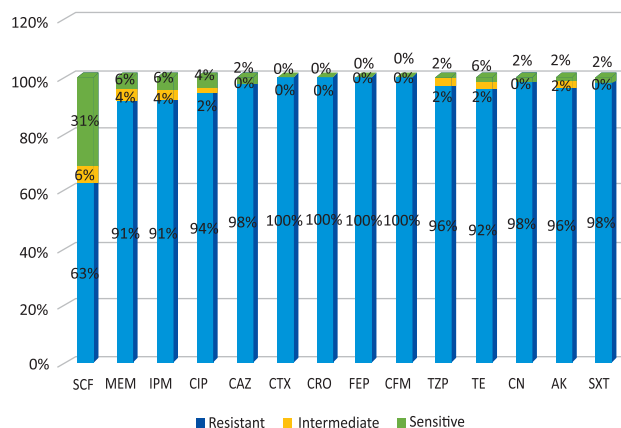


Figure 3: Antimicrobial Susceptibility Pattern of Isolated *Acinetobacter* Species

## DISCUSSION

Antimicrobial resistance is one of the greatest threats to human health globally.<sup>19</sup> The increase in the prevalence of *Acinetobacter* species is alarming due to narrow therapeutic range attributed to its multidrug resistant pattern.<sup>20</sup>

The prevalence of *Acinetobacter* species in our study was low. Out of the 2490 culture specimens, 51(2%) were positive for *Acinetobacter* species. Similar results were reported in another study conducted in Saudi Arabia in which *Acinetobacter* species were found in 166(1.5%) specimens out of the total 10852 blood culture samples.<sup>4</sup> In contrast, the prevalence of *Acinetobacter* species was high in other studies. The prevalence of *Acinetobacter* species was 38% in the United States (from 346 states),<sup>21</sup> 4.7% in India,<sup>22</sup> 4.3% in South Africa,<sup>23</sup> 7.3% in Postgraduate Medical Institute, Lahore<sup>20</sup> and 9.2% in Morocco.<sup>24</sup>

Fifty nine percent of the patients from which *Acinetobacter* species were isolated were females and 41% were males. Study by Wisplinghoff et al., 58% of the patients with *Acinetobacter* infection were men.<sup>4</sup> In a study by Aneela et al., 61.5% of patients were males and 38.5% were females.<sup>20</sup> In our study, only 7(14%) patients were below 1 year of age, 5(10%) patients were between 1 to 18 years, 24(47%) patients were between 19 to 50 years and 15(29%) patients were above 50 years. In another study, most of the patients from which *Acinetobacter* was isolated were from the age group 0-29 days (42.9%) followed by age groups 40-60 years (18.7%) and 1-20 years (15.4%).<sup>1</sup>

The various clinical specimens from which *Acinetobacter* species were isolated were 34(66.7%) pus/wound swabs followed by 8(15.7%) blood, 3(5.9%) urine, 3(5.9%) tips (2 center venous catheters, 1 endotracheal tube), 2(3.9%) sputum and 1(1.9%) CSF. According to a study conducted in different tertiary care hospitals of Lahore, Pakistan during 2013-2014, the majority of the *Acinetobacter* species were isolated from pus samples (31.3%) followed by CSF (25%), blood (17.5%), CVP tips (13.7%), wound swabs (5%). One isolate was recovered from each of the following samples including urine, high vaginal swab (HVS), sputum, pleural fluid, throat and tracheostomy secretions (1.25%).<sup>25</sup> A study conducted at Pakistan Institute of Medical Sciences (PIMS) showed that most of the *Acinetobacter* species were isolated from endotracheal tubes secretions (25.3%) and tracheal secretions (19.8%) with the minimum number of strains from pus (16.5%).<sup>1</sup>

Sixty four percent *Acinetobacter* species were isolated from outpatient departments followed by nursery (14%), ICU (8%), surgery (6%), medicine (2%), neurosurgery (2%), obstetrics & gynecology (2%) and pediatrics (2%). In contrast in a study by Sherwani et al., the majority of the *Acinetobacter* species were

isolated from intensive care units (44%), surgery (19%), medicine (15%), orthopaedics (11%), burn unit (4%), paediatrics (3%), gynaecology (2%) and outpatient departments (2%).<sup>22</sup> *Acinetobacter* species were isolated from nephrology (22.3%), cardiology (18.3%), surgery (13.7%), ICU (10.6%), oncology (10.6%), medicine (10.6%), neonatal unit (7.7%) and neurology (6.2%) in Children Hospital, Lahore.<sup>26</sup> In another study, most of the *Acinetobacter* strains were recovered from surgical wards (36.3%), medicine (20.1%), orthopedics (14.1%), gynaecology (12%), neurology (9%) and pediatrics (8.5%).<sup>21</sup> According to a study by Begum et al., the highest frequency of *Acinetobacter* were recovered from neonatal intensive care unit (42.9%) and medical ICU (19.8%) and the minimum number from the outpatient department (9.9%).<sup>1</sup>

In our study, 31% strains were sensitive to cefoperazone-sulbactam. Only 6% of strains were sensitive to tetracycline and 2% strains were sensitive to ceftazidime, tazocin, trimethoprim-sulfamethoxazole and aminoglycosides (gentamycin, amikacin). Carbapenem and ciprofloxacin resistance was seen in 91% and 94% strains respectively. All of the strains were resistant to cephalosporins (cefotaxime, cefepime, ceftriaxone and cefixime). This showed that most of the strains were resistant to a number of antimicrobials. Out of the 37 positive samples of pus/wound and catheter tips, 14(37.8%) were sensitive and 23(62.2%) were resistant to tigecycline.

Almost the same antimicrobial resistance pattern was observed in a study by Kettani et al. which showed that 98.6% and 85.4% *Acinetobacter* species were resistant to cefotaxime and ceftazidime. The resistance to ciprofloxacin, gentamicin, imipenem and amikacin was observed in 88.2%, 78.1%, 75.7% and 63.5% isolates respectively.<sup>24</sup> In a study by Khawaja et al., most of the strains were sensitive to doxycycline (62.8%) followed by tigecycline (59.8%) and tazocin (55.1%). Ninety two percent strains were resistant to sulbactam. Among the cephalosporins, 89.7% and 81.2% were resistant to ceftriaxone and cefotaxime respectively. Sixty nine percent strains were resistant to amikacin & gentamicin, 70.1% to ciprofloxacin, 68.4% to aztreonam and 66.7% to tetracycline.<sup>20</sup> A study carried out at Chughtai Laboratory, Lahore revealed 716 *Acinetobacter* isolates. Out of 716 strains, 99.2% were resistant to cefotaxime & ceftazidime whereas 93.6% and 90.9% were resistant to gentamicin and imipenem respectively. However, all of them were sensitive to colistin, polymyxin B and tigecycline.<sup>27</sup> Begum et al. reported 100% resistance of *Acinetobacter* strains to cephalosporins, carbapenems, fluoroquinolones and  $\beta$ -lactam drugs in her study. Sixty (65.9%) strains were resistant to tetracycline. All the strains were sensitive to tigecycline and minocycline.<sup>1</sup> Saba and colleagues

reported that the maximum resistance (98.8%) was shown to cephalosporins followed by trimethoprim-sulphamethoxazole (98.43%), aztreonam (88.8%), gentamicin (86.3%), imipenem (77.5%), piperacillin-tazobactam (72.5%) and doxycycline (72.1%).<sup>25</sup> About 52.5% strains were sensitive to tigecycline.

Surprisingly, in another study 100% *Acinetobacter* strains were inhibited by imipenem followed by 96% and 91% isolates sensitive to amikacin and doxycycline respectively. About 58% strains were sensitive to ciprofloxacin, 62% to piperacillin, 64% to ceftazidime and 72% to cefepime.<sup>4</sup> In India, the antimicrobial resistance of *Acinetobacter* strains to piperacillin, ceftazidime, amikacin, cefepime, imipenem and ciprofloxacin was seen in 49%, 48%, 46%, 41%, 33% and 27% respectively.<sup>22</sup>

In our study, multidrug resistance was seen in all 51 *Acinetobacter* strains and 5(9.8%) strains of *Acinetobacter* species were resistant to all of the antimicrobials. A study by Wisplinghoff et al. demonstrated that 30% of *Acinetobacter* isolates were multidrug resistant.<sup>4</sup>

Susceptibility pattern of pathogens has been changing over the years implying the need for periodic monitoring in order to decrease the number of therapeutic failures and boost an effort to arrest the growing occurrence of antibiotic resistance. Microbiologists have to work in collaboration with clinicians in installing newer and appropriate antibiotic discs according to emerging resistance patterns and antibiogram

### CONCLUSION

The prevalence of *Acinetobacter* species was low (2%) in different clinical specimens for culture and sensitivity in Sharif Medical City Hospital, Lahore. Thirty one percent strains were sensitive and 6% were intermediate to cefoperazone-sulbactam. All the strains of *Acinetobacter* species were multidrug resistant. Five (9.8%) strains of *Acinetobacter* species were resistant to all of the antimicrobials. However, the emergence of infections by multidrug resistant *Acinetobacter* species is an alarming sign in the hospital setting.

### RECOMMENDATIONS

- The widespread and unnecessary use of antimicrobials should be avoided so as to reduce the emergence of multidrug resistant organisms.
- As *Acinetobacter* is resistant to most of the antimicrobials, further work should be done to determine its sensitivity pattern to colistin and polymyxin B by using minimum inhibitory concentration (MIC) according to CLSI and Eucast.
- The use of colistin and polymyxin B should be

reserved only for *Acinetobacter* strains resistant to all other antimicrobials.

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