

Changing Antimicrobial Susceptibility and Resistance Pattern of Acinetobacter Species over the Last Eight Years in a Tertiary Care Hospital in Lahore, Pakistan

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ABSTRACT

Background: Acinetobacter spp. is a highly resistant nosocomial pathogen that leads to a broad range of human infections resulting in high morbidity and mortality. Due to unpredictable MDR patterns of Acinetobacter spp., it is imperative to know the institutional prevalent susceptibility profiles of these residing pathogens. The objective of this study was to determine the antimicrobial susceptibility pattern of Acinetobacter species over the last 8 years in a tertiary care hospital in Lahore, Pakistan.

Material and Methods: A retrospective study was carried out in Lahore General Hospital, a tertiary care hospital in Lahore, Pakistan. Eight-year data was gathered from January 2012 to December 2019. All specimens were handled according to standard operating procedures in the microbiology laboratory of the Pathology department of Lahore General Hospital. The Acinetobacter spp. were identified in the laboratory by Gram staining, oxidase test, catalase test and Triple sugar iron fermentation and their antibiotic sensitivity pattern was noted.

Results: The highest yield of Acinetobacter spp. from the clinical specimen was isolated from pus followed by tracheal secretion, blood, and urine in the last three years (from 2017 to 2019). Most of the isolates were multi-drug resistant (MDR). There was a progressive increase in resistance of Acinetobacter spp. The highest progression in resistance was observed among the cephalosporin and quinolone group of antibiotics.

Conclusions: Increased resistance to commonly used antimicrobials against Acinetobacter species has been observed with the highest resistance to quinolones and cephalosporins.

Key words: Acinetobacter, Antibiotic stewardship, Multidrug resistance, Nosocomial infections.

Authors' Contribution:

¹Conception; Literature research; manuscript design and drafting; ^{2,3}Critical analysis and manuscript review; ⁴⁻⁶Data analysis; Manuscript Editing.

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Introduction

Acinetobacter species is one of the most important opportunistic non-fermenting bacteria associated

with hospital-acquired infections with almost 20% of infections occurring in intensive care units.¹ It is a

major cause of urinary tract infection, surgical site infection, septicemia, pneumonia, and ventilator-associated pneumonia. In the past, they were considered contaminants but now they are an important cause of healthcare-associated infections.² Hospital-acquired infections are the sixth leading cause of death in the USA.³ Invasive procedures and misuse of broad-spectrum antibiotics are the major risk factors for the acquisition of infection with *Acinetobacter* species. Increased resistance leads to treatment failure, prolonged hospital stays, financial burden and increased mortality.^{4,5}

Multidrug efflux pumps, target modification, permeability defects, and enzymatic degradation of drugs are important mechanisms of acquired resistance against a wide range of antibiotics.⁶ *Acinetobacter* species have been declared by WHO as one of the most serious organisms among ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter* species, *Pseudomonas aeruginosa*, and *Enterobacter* species).⁶

It is very difficult to eradicate *Acinetobacter* from the environment due to its ability to survive on dry surfaces for a longer period and resistance to most of the disinfectants.⁷ About 48-85% of the clinical isolates have shown multidrug resistance.¹ *Acinetobacter* spp. are emerging as one the most resistant pathogens in Pakistan as well as globally and are a great burden to human health.^{8,9}

Increased incidence of MDR strains of *Acinetobacter* species is due to a lack of compliance with basic infection control measures.⁸ Due to unpredictable MDR patterns of *Acinetobacter* spp., it is imperative to know the institutional prevalent susceptibility profiles of these residing pathogens. Hence, this study was conducted to observe the antibiogram of the last eight years of various clinical samples from which *Acinetobacter* spp. was isolated by a

simplified phenotypic identification protocol and to determine the changing antibiotic susceptibility pattern of these isolates. This is essential for proper antimicrobial stewardship, which is the key to effective control of infections.

Material and Methods

This retrospective study was carried out in Lahore General Hospital, Lahore, a tertiary care hospital. Approval was obtained from the Ethical Review Committee of Postgraduate Medical Institute/ Ameer-ud-Din Medical College/ Lahore General Hospital, Lahore, Pakistan. This study comprised of eight-year data from January 2012 to December 2019.

Samples from both indoor and outdoor patients were collected and processed according to standard protocols of the microbiology laboratory of the Pathology department, Lahore General Hospital, Lahore, Pakistan. These samples included blood, CSF, pus, wound swabs, tips, tracheal secretion, fluid, sputum, bronchial wash, pleural tap, tissue/biopsy, and urine. All *Acinetobacter* spp. isolated and identified after biochemical tests were included in this study.

Clinical samples from patients already taking antibiotics and repeat isolates from the same patient were excluded.

All clinical specimens were initially inoculated on a Blood agar and MacConkey agar, while the urine specimens were inoculated on Cystine Lactose Electrolyte Deficient (CLED) medium. The inoculated plates were incubated aerobically at 37°C and checked for bacterial growth after 24 hours. The morphology of bacterial colonies was examined with a hand lens. The bacteria were identified first by Gram staining, catalase test, oxidase tests, and hanging drop method for motility. The *Acinetobacter* spp. were identified as Gram-negative or Gram variable coccobacilli, oxidase

negative and catalase positive. Each strain was inoculated on the Triple sugar iron (TSI) slant for the identification of its ability to ferment sugars.

The antimicrobial susceptibility pattern was evaluated by the Kirby-Bauer disc diffusion method according to respective CLSI guidelines.¹⁰ A bacterial suspension of 0.5 McFarland turbidity standard was made and inoculated on Muller Hinton agar plate for each *Acinetobacter* spp. isolate. Aminoglycosides, Carbapenem, Quinolones, Cephalosporins, Ampicillin / Sulbactam, Cotrimoxazole, Piperacillin / Tazobactam, and Doxycycline discs were inoculated on plates and incubated for further 24 hours of aerobic incubation at 37°C.

ATCC 19606 strain of *Acinetobacter* species was used as a quality control strain in all standard operating procedures.¹¹ Frequencies and percentages were calculated for the qualitative variables like *Acinetobacter* species isolated from clinical samples and percentage resistance pattern to various antibiotics.

Results

The results were tabulated according to each year. A total number of clinical samples were tabulated from which *Acinetobacter* species were isolated according to the specimen.

The highest yield of *Acinetobacter* spp. from the clinical specimen was isolated from pus followed by tracheal secretion, blood, and urine in the last three years from 2017 to 2019 (Table I).

Figure 1 is showing the eight years resistance pattern in our settings with a progressive increase in resistance of *Acinetobacter* spp. The highest progression in resistance was observed among the cephalosporin and quinolone groups of antibiotics

Discussion

Despite rigorous endeavors, nosocomial multi-drug resistant (MDR) *Acinetobacter* spp. is still a major problem owing to its great capability to propagate and colonize human and environmental reservoirs. *Acinetobacter* spp. is a highly resistant nosocomial pathogen that leads to a broad range of human infections resulting in high morbidity and mortality rates.¹² Over the years *Acinetobacter* species are increasingly being isolated from a broad array of clinical specimens. It is the need of the day to periodically analyze the antimicrobial susceptibility and resistance pattern of this common pathogen so that appropriate antibiotic policy can be formulated for empirical as well as a targeted treatment.

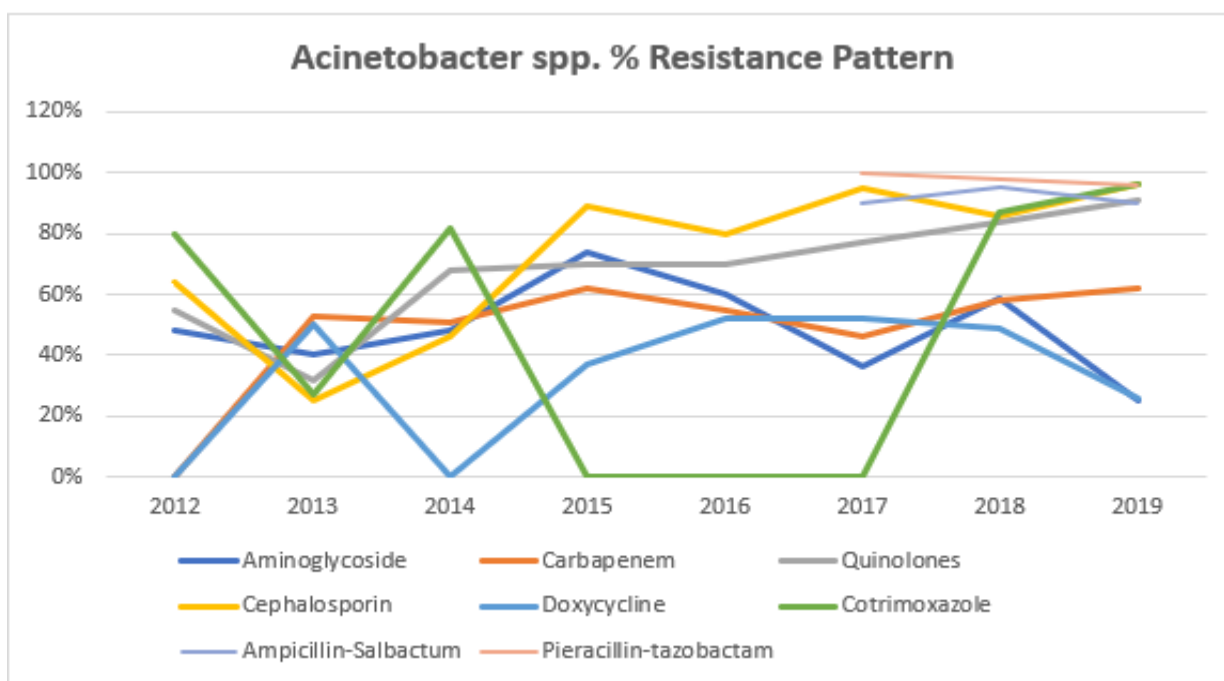
The variations in the antibiogram of *Acinetobacter* spp. demands periodic surveillance of these pathogens for an appropriate selection of therapy.¹³

Acinetobacter species has attained a foothold in the ICU settings, causing nosocomial pneumonia which accounts for around 36% of hospital-acquired pneumonia in Asian countries.^{14,15} This is in concordance with this study, which shows *Acinetobacter* species infections on the rise over the past eight years, especially in the ICUs. This is an imminent threat for the spillover of this organism in the general wards, highlighting the importance of keeping a strict eye over its prevalence and antimicrobial susceptibility and resistance pattern.

Analysis of antibiograms of *Acinetobacter* species over the last eight years in our hospital has unveiled some interesting findings. An increased pattern of antimicrobial resistance has been reported in a tertiary care hospital in Nepal with 100% resistance to cephalosporins.¹⁶ These observations indicated that cephalosporins are no more a drug of choice for effective treatment of *Acinetobacter* infections.¹⁷ This is an alarming situation as our study also shows cephalosporin resistance on the rise over the last

Specimen	2012	2013	2014	2015	2016	2017	2018	2019	n
Blood	28	22	29	38	32	152	161	257	719
CSF	7	31	27	30	42	61	98	187	483
Pus	44	48	54	57	56	198	390	407	1245
Tracheal secretion	--	2	1	17	32	207	312	478	1049
Sputum	--	7	3	--	4	30	37	42	123
Wound swab	--	5	2	28	24	69	85	76	289
Bronchial wash	--	--	--	--	8	8	7	5	28
Fluid	--	--	--	9	8	19	13	25	74
Tips	--	4	2	17	48	76	95	76	218
Pleural tap	--	--	--	--	--	2	4	7	13
Urine	11	6	15	38	92	143	170	206	681
Tissue/ biopsy	--	--	--	--	2	21	30	56	109
Total organisms isolated	90	126	133	234	348	986	1438	1822	5140

n-total number of specimens; CSF-Cerebrospinal fluid



* 0% values show the antibiotic discs were not available in these years

Figure 1: Eight-year percentage resistance pattern of different groups of Antibiotics to *Acinetobacter spp.*

eight years with resistance reaching up to almost 100%. The carbapenems are considered salvage therapy for most multidrug-resistant organisms. However, the emergence of carbapenemase enzymes is a worldwide phenomenon narrowing the available spectrum of antimicrobials for the treatment of MDR *Acinetobacter* species.¹⁸ The

present study showed approximately 60% resistance to carbapenems which are also very alarming for clinically isolated *Acinetobacter spp.* in the tertiary care hospital.

Another alarming finding is the rise of antimicrobial resistance to quinolones reaching up to 90% in the

year 2019. Elshahat et al. and Roy et al. commented in their recent studies on quinolones resistance in clinical isolates of *Acinetobacter* spp. According to them, quinolones resistance is more than 90% among *Acinetobacter* isolates, which is consistent with the present study.^{19,20}

An interesting finding of our study is a revival of antimicrobial susceptibility to Doxycycline. Sadhu et al. reported that Doxycycline has good efficacy against MDR *Acinetobacter* spp., and its use can be considered as an alternative therapy to down-regulate the pressure on other groups of antibiotics.²¹ Therefore, the sensitive pattern of doxycycline in our hospital settings would be a good sign for treating nosocomial infections.

This may be due to the phenomenon of antibiotic holidays and holds a promising perspective for other antibiotics as well. This can contribute to the appropriate institution of antimicrobial stewardship which involves giving the right antibiotic, at the right time, in the right dose, and for an appropriate period. Observing antibiotic holidays stepwise for other groups of antibiotics may lead to a revival of their susceptibility and hence clinical effectiveness.

The progressively increasing trend of antimicrobial resistance in *Acinetobacter* species to major groups of antibiotics over the last eight years is an eye-opener for our hospital as well as for antibiotic policymaking. It is high time that all tertiary care hospitals maintain and publish yearly antibiograms, as this is an essential component of antimicrobial stewardship.

Conclusion

The current antibiogram of *Acinetobacter* species revealed the progressively increasing resistance of this hospital bug which is now posing a great threat to patients in hospital settings. Judicial use of antibiotics by clinicians should be ensured to maintain an antibiotics stewardship program.

Recommendations

We recommend every tertiary care hospital maintain its hospital antibiograms. This will help to maintain antibiotic stewardship in their hospital settings according to their local trend of antibiotic resistance. We also recommend following strict infection prevention and control programs to overcome antibiotic resistance among local hospital nosocomial bacteria.

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