ORIGINAL ARTICLE

IN-VITRO ASSESSMENT OF THE THERAPEUTIC POTENTIAL OF POLYMYXINS AND TIGECYCLINE AGAINST MULTIDRUG-RESISTANT *ACINETOBACTER* ISOLATES FROM INFECTED WOUNDS

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Background: The incidence of multidrug-resistant (MDR), extreme drug resistant (XDR), and pan drug-resistant (PDR) *Acinetobacter* are increasing throughout the world. The therapeutic management and control of *Acinetobacter* are difficult due to the emergence of drug resistance and its enduring capacity to survive in the environment. The present study was designed to appraise the efficacy of Polymyxins and Tigecycline against multidrug-resistant *Acinetobacter* isolates from surgical and burn wounds. **Methods:** During the study, the specimens were collected from various types of wounds from inpatients and outpatients of the tertiary care hospitals of Lahore, Pakistan in 2017 and 2018. The bacterial pathogens were isolated and identified using standard microbiological procedures and molecular confirmation of Acinetobacter species was examined by PCR using specific primers. The antibiotic susceptibility profiling of *Acinetobacter* isolates was studied against 18 antibiotics as per Clinical and Laboratory Standards Institute (CLSI) guidelines. **Results:** The *Acinetobacter* isolates demonstrated extreme resistance especially to ampicillin/sulbactam, piperacillin/tazobactam, cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides. However, the colistin, polymyxin, and tigecycline remained the most effective antimicrobial agents against *Acinetobacter* isolates. **Conclusion:** The results highlight the extent of drug resistance and therapeutic potential of Polymyxins and Tigecycline for wound infections caused by MDR and XDR *Acinetobacter* species. The wiser use of antimicrobials, incessant surveillance of antimicrobial resistance, and stringent adherence to infection control guidelines are critical to reducing major outbreaks in the future.

Keywords: *Acinetobacter*; Wounds; Antimicrobial resistance; Polymyxins; Tigecycline

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INTRODUCTION

Acinetobacter is an opportunistic Gram-negative bacterium that has gained importance through a range of community and hospital-acquired infections. The management of infections caused by multidrug-resistant *Acinetobacter* is challenging. Although the antimicrobial resistance among *Acinetobacter* isolates has been increasing, however, definitions of multidrug resistance differ in the literature.[1,2] Multidrug-resistant *Acinetobacter* is an emerging pathogen in the health care facilities that can cause multiple infections including pneumonia, meningitis, bacteremia, urinary tract infection, and wound infections. The ability of this pathogen to endure wider environmental conditions and its persistence for long periods on surfaces enables it to be a frequent cause of outbreaks.[3,4] Antimicrobial resistance to commonly used antimicrobials is another reason for the spread of this pathogen.[5,6] Various aspects of Acinetobacter infections are still unclear especially the epidemiological and management issues. There is ongoing controversy regarding mortality in ICU patients that are directly attributable to *Acinetobacter* infections.[7,8] However, it is evident from the literature that this pathogen may cause life-threatening infection thereby contributes towards substantial mortality among the patients.[7]

The development of wound infection depends on the composite interaction of multiple factors. Increased numbers of various cell types enter the wound and initiate an inflammatory response that is characterized by classical signs of pain, redness, swelling, and increased temperature. Wound infection is the successful assault and proliferation by one or more species

of microorganisms within the tissues and may result in pus formation.[9]

The antimicrobial resistance among *Acinetobacter* species is increasing with the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan drug-resistant (PDR) isolates. Although very less data is available from Pakistan regarding the antimicrobial resistance status of this emerging pathogen, the few published studies reported the resistance to almost all antibiotics commonly prescribed by the physicians including cephalosporins, carbapenems, aminoglycosides, tetracycline, and fluoroquinolones. In most developing countries, the lack of facilities, postoperative care, and patient's compliance with antibiotic therapy leads to infections in surgical and burn wounds. The study aimed to determine the susceptibility patterns of multidrug-resistant *Acinetobacter* isolates from wound samples and assessment of Polymyxins and Tigecycline against these "superbugs".

MATERIAL AND METHODS

The antibiotic resistance of 204 *Acinetobacter* isolates from wound samples was studied during 2017 and 2018. The samples were cultured on MacConkey agar, Blood agar, and Chocolate agar and incubated for 24 hours at 37 °C initially and further 48 hours in case of no growth. The cultures were processed and initially identified by standard microbiological procedures.[10]

The molecular characterization of Acinetobacter strains was performed through amplification of 425-bp region of the *rec*A gene of *Acinetobacter* using specific primers, forward 5'-CCTGAATCTTCTGGTAAAAC-3' and reverse 5'-GTTTCTGGGCTGCCAAACATTAC-3'as described previously. [11] The PCR reaction was performed in a total reaction volume of 25 µl containing 12.5 µl master mix (Thermo Fisher Scientific, USA). PCR conditions comprised 94 °C for 10 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 seconds and 72 °C for 30 seconds, and a final extension at 72 °C for 10 min. The amplicons were examined by electrophoresis on 1% agarose gels. The PCR products were sequenced from Macrogen® (South Korea) and nucleotide sequence homology was examined using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Antimicrobial susceptibility was performed by the Kirby-Bauer disk diffusion method as per CLSI guidelines. Commercially available antibiotic discs such as Ampicillin/sulbactam (10/10 µg), Piperacillin/Tazobactam (100/10 µg), Cefepime (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Imipenem (10 µg), Meropenem (10 µg), Amikacin (30 µg), Gentamicin (10 µg), Tobramycin (10 µg), Doxycycline (30 µg), Tigecycline, Ciprofloxacin (5 µg), Levofloxacin (5 µg) and Co-trimoxazole (1.25/23.75 µg).

The MIC of Colistin, Polymyxin B, and tigecycline was performed by the broth microdilution methods as per CLSI guidelines. The bacterial strains were inoculated in Mueller Hinton broth and incubated at 37 °C for 18 hours. The broth culture was adjusted for turbidity using a 0.5 McFarland turbidity standard. *E. coli* (ATCC 25922) was used as reference strains for quality control. The MIC results were interpreted according to the CLSI guideline.[12]

MDR, XDR, and PDR strains were classified according to the criteria defined by the Centers for Disease Control and Prevention (CDC) and the European Centre for Disease Prevention and Control (ECDC).[13] Briefly, the isolates were defined as MDR if resistant to at least one agent in three or more antimicrobial categories, XDR if resistant to at least one agent in all but susceptible to one or two antimicrobial categories, and PDR if resistant to all agents in all antimicrobial categories.

RESULTS

During the study, 204 *Acinetobacter* isolates were obtained from wound samples including 112 (54.90 %) from male and 92 (45.10 %) from female patients. The median age of the patients was 44 years ranging from 10 months to 88 years.

The overall antimicrobial resistance pattern showed that *Acinetobacter* isolates were found highly resistant to Ampicillin/sulbactam (100%) and third-generation cephalosporins, i.e., ceftazidime (99.51%), ceftriaxone (99.51%), and cefotaxime (99.51%). High rates of resistance also demonstrated to cefepime (97.55%), ciprofloxacin (97.55%), amikacin (85.78%), and gentamicin (93.63%). However, relatively less resistance was observed against Tobramycin (66.67%) and Doxycycline (69.12%). Among the two hundred and four *Acinetobacter* isolates, 87.25% were resistant to Carbapenems (imipenem and meropenem). Out of 204 *Acinetobacter* isolates, 202 (99%) and 178 (87%) were categorized as MDR and XDR, respectively (Figure-2).

Tigecycline, colistin, and polymyxin B were the most effective antimicrobial agents as none of the *Acinetobacter* isolates was resistant to

Tigecycline, and Polymyxin B, while only 1/204 (0.49 %) isolate was resistant to colistin. The distribution of MIC for polymyxin B, colistin, and tigecycline is shown in table-3. Small variations were observed in the antimicrobial resistance pattern among *Acinetobacter* isolates obtained from the patients of different age groups. The percent resistance against penicillin combinations and different generations of cephalosporin was found highest in the age group (21–40). patients whereas relatively low in age groups less than 20 and more than 60. Percent resistance against ciprofloxacin and cotrimoxazole was also high (100% and 98.61%) among the age group 21–40. The Higher rate of resistance against Amikacin and Gentamicin (8.89% and 85.71%, respectively) was also observed in the age group 21–40 as compared with other groups. However, the resistance rate against Imipenem was raised with age, lowest (78.26%) in the age group less than 20, and highest (89.74%) among the patients of age group more than 60. The trends of antibiotic resistance for *Acinetobacter* isolates among different age groups are shown in figure-1.

Table-1: Relative frequency distribution of *Acinetobacter* among different age groups in male and female patients

Age Groups (Years)	Male	Female	Total
< 20	16	7	23
21–40	43	29	72
41–60	33	37	70
> 60	20	19	39
Total	**112**	**92**	**204**

Table-2: Antibiogram of *Acinetobacter* species from wound samples

Antimicrobial Agents	Male (112)				Female (92)				Total (204)			
	Sensitive		Resistant		Sensitive		Resistant		Sensitive		Resistant	
	n	%	n	%	n	%	n	%	n	%	n	%
Ampicillin/sulbactam	0	0	112	100	0	0	92	100	0	0	204	100
Piperacillin/tazobactam	15	16.07	94	83.93	15	16.3	77	83.7	33	16.18	171	83.82
Cefepime	3	2.68	109	97.32	2	2.17	90	97.83	5	2.45	199	97.55
Cefotaxime	1	0.89	111	99.11	0	0	92	100	1	0.49	203	99.51
Ceftazidime	1	0.89	111	99.11	0	0	92	100	1	0.49	203	99.51
Ceftriaxone	1	0.89	111	99.11	0	0	92	100	1	0.49	203	99.51
Imipenem	19	16.96	93	83.04	7	7.61	85	92.39	26	12.75	178	87.25
Meropenem	19	16.96	93	83.04	7	7.61	85	92.39	26	12.75	178	87.25
Amikacin	19	16.96	93	83.04	10	10.87	82	89.13	29	14.22	175	85.78
Gentamicin	8	7.14	104	92.86	5	5.43	87	94.57	13	6.37	191	93.63
Tobramycin	36	32.14	76	67.86	32	34.78	60	65.22	68	33.33	136	66.67
Doxycycline	35	31.25	77	68.75	28	30.43	64	69.57	63	30.88	141	69.12
Ciprofloxacin	3	2.68	109	97.32	2	2.17	90	97.83	5	2.45	199	97.55
Levofloxacin	3	2.68	109	97.32	2	2.17	90	97.83	5	2.45	199	97.55
Co-trimoxazole	7	6.25	105	93.75	7	7.61	85	92.39	14	6.86	190	93.14
Colistin	112	100	0	0	91	98.91	1	1.09	203	99.51	1	0.49
Polymyxin B	112	100	0	0	92	100	0	0	204	100	0	0
Tigecycline	112	100	0	0	92	100	0	0	204	100	0	0

Table-3: MIC distribution of 204 *Acinetobacter* isolates

Antimicrobial Agents	MIC Breakpoints (µg/mL)	No. of isolates for which the MIC (µg/mL) were										
		0.125	0.25	0.5	1	2	4	8	16	32	64	128
Polymyxin B[a]	≥4	21	48	91	42	2	-	-	-	-	-	-
Colistin[a]	≥4	9	32	105	53	4	1	-	-	-	-	-
Tigecycline[b]	≥8	18	59	103	21	3	-	-	-	-	-	-

[a]Based on CLSI (2015) breakpoints, [b]Based on FDA breakpoints for tigecycline susceptibility

Table-4: Antimicrobial resistance profile of the *Acinetobacter* isolates

Antimicrobial resistance profile	No of isolates
AK/IMP/CIP/TZP/CAZ/SXT	153 (75.0)
AK/IMP/CIP/TZP/CAZ/SAM	162 (79.4)
AK/IMP/CIP/TZP/SXT/SAM	153 (75.0)
AK/IMP/CIP/CAZ/SXT/SAM	167 (81.9)
AK/IMP/TZP/CAZ/SXT/SAM	153 (75.0)
AK/CIP/TZP/CAZ/SXT/SAM	160 (78.4)
IMP/CIP/TZP/CAZ/SXT/SAM	153 (75.0)
AK/IMP/CIP/TZP/CAZ/DO	114 (55.9)
AK/IMP/CIP/TZP/SXT/DO	106 (52.0)
AK/IMP/CIP/CAZ/SXT/DO	116 (56.9)
AK/IMP/TZP/CAZ/SXT/DO	106 (52.0)
AK/CIP/TZP/CAZ/SXT/DO	109 (53.4)
IMP/CIP/TZP/CAZ/SXT/DO	106 (52.0)
AK/IMP/CIP/TZP/SAM/DO	114 (55.9)
AK/IMP/CIP/CAZ/SAM/DO	126 (61.8)
AK/IMP/TZP/CAZ/SAM/DO	126 (61.8)
AK/CIP/TZP/CAZ/SAM/DO	117 (57.4)
IMP/CIP/TZP/CAZ/SAM/DO	114 (55.9)
AK/IMP/CIP/SXT/SAM/DO	116 (56.9)
AK/IMP/TZP/SXT/SAM/DO	106 (52.0)
AK/CIP/TZP/SXT/SAM/DO	109 (53.4)
IMP/CIP/TZP/SXT/SAM/DO	106 (52.0)
AK/IMP/CAZ/SXT/SAM/DO	116 (56.9)
AK/CIP/CAZ/SXT/SAM/DO	129 (63.2)
IMP/CIP/CAZ/SXT/SAM/DO	116 (56.9)
AK/TZP/CAZ/SXT/SAM/DO	109 (53.4)
IMP/TZP/CAZ/SXT/SAM/DO	106 (52.0)
CIP/TZP/CAZ/SXT/SAM/DO	109 (53.4)
AK/IMP/CIP/TZP/CAZ/SXT/DO	106 (52.0)
AK/IMP/CIP/TZP/CAZ/SXT/SAM	153 (75.0)
AK/IMP/CIP/TZP/CAZ/SAM/DO	114 (55.9)
AK/IMP/CIP/TZP/SXT/SAM/DO	106 (52.0)
AK/IMP/CIP/CAZ/SXT/SAM/DO	116 (56.9)
AK/IMP/TZP/CAZ/SXT/SAM/DO	106 (52.0)
AK/CIP/TZP/CAZ/SXT/SAM/DO	109 (53.4)
IMP/CIP/TZP/CAZ/SXT/SAM/DO	106 (52.0)
AK/IMP/CIP/TZP/CAZ/SXT/SAM/DO	106 (52.0)

AK; Amikacin, IMP; Imipenem, CIP; Ciprofloxacin, TZP; Tazobactam-Piperacillin, CAZ; Ceftazidime, SXT; Trimethoprim-Sulfamethoxazole, SAM; ampicillin-sulbactam, DO; Doxycycline

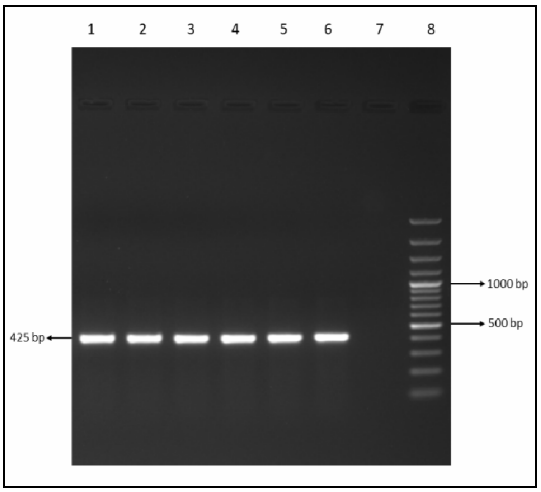

Figure-1: PCR product after agarose gel electrophoresis. Lane 1-6, PCR Products with recA specific primers (425 bp); Lane 7, *E. coli* as negative control; Lanes 8 GeneRuler 100 bp DNA Ladder

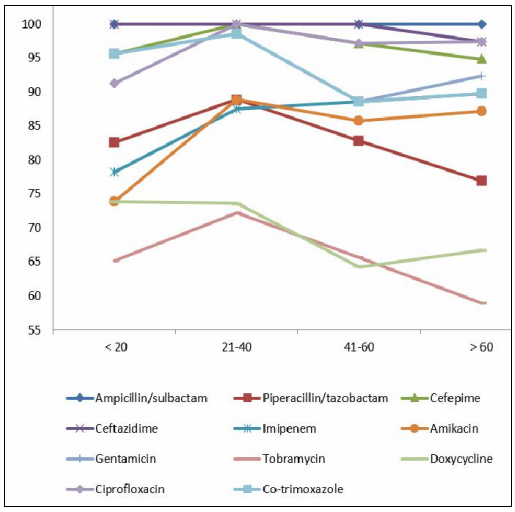

Figure-2: Age-wise distribution of the resistance pattern of Acinetobacter isolates to various antimicrobial agents

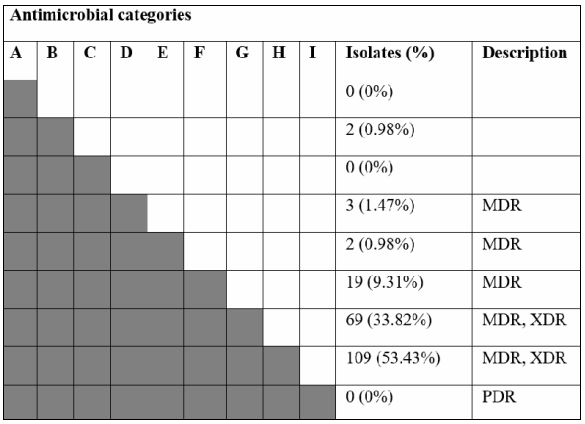

Figure-3: Random distribution of *Acinetobacter* isolates according to the definition of MDR, XDR, and PDR

DISCUSSION

The emergence and widespread of *Acinetobacter* species especially resistant to most of the available antibiotics is an area of immense apprehension. *Acinetobacter* species are now being commonly associated with many hospital-associated infections**.** The Management of infections caused by MDR and XDR *Acinetobacter* species are a big challenge for physicians as well as for clinical microbiologists. The ability of *Acinetobacter* to survive in healthcare settings and its capacity to persevere for long periods over the surfaces enables it to be a frequent and recurrent cause of hospital-associated infections that lead to multiple outbreaks.[14,15]

The prevalence of Acinetobacter from pus/wound samples has shown variation in different studies ranging from 11.7–27.5%.[16] The prevalence of *Acinetobacter* from positive pus samples was observed as 2.46% in our study. The difference in the prevalence of *Acinetobacter* species is mainly due to disparities in the identification system especially when conventional processes of identification are used.[17]

The obtained isolates showed high resistance to ampicillin/sulbactam (100%), Piperacillin/tazobactam (83.82%), ceftazidime (99.51%), amikacin (85.78%), gentamicin (93.63%), cotrimoxazole (93.14%), and ciprofloxacin (97.55%). The results are comparable to a recent study in Iran that reported the resistance rate of Acinetobacter isolates to Piperacillin/tazobactam (96.1%), ceftazidime (96.1%), amikacin (88.8%), gentamicin (83%), cotrimoxazole (89.8%) and ciprofloxacin (96.6%).[18] In the present study, we found that almost all the Acinetobacter isolates were found susceptible to colistin, polymyxin B, and tigecycline. Similar results were reported in a recent study.[18] The increased emergence of resistance among *Acinetobacter* species to routinely used antimicrobials necessitate the introduction of other non-antibiotic means of treatment like herbal drugs, medicinal plants, and phage therapy.

Carbapenems, tetracycline, and Polymyxin were considered as the most effective antimicrobial agents against *Acinetobacter* in the past.[19,20] However carbapenems resistant strains are becoming increasingly prevalent therefore limiting the therapeutic options for this organism.[21] The rate of resistance to imipenem was 87.25 % in the present study. Lower rates of Carbapenem resistance of *Acinetobacter* species were reported during the past years in some parts of the world such as Taiwan (10%), and Japan (3.2%).[22,23] Antimicrobial susceptibility of 490 *Acinetobacter* isolates collected from multicenter in 11 European countries from 1997 to 2000, the resistance rate for imipenem and meropenem was 16% and 18% respectively.[24] A data for 2006 from 40 centers of 12 European countries participating in the monitory program demonstrated a significant rise in resistance rates for imipenem (42.5%) and meropenem (43.4%).[25] Another study revealed that the resistance rate among Acinetobacter was increased from 0% to 42% for imipenem during the study period.[26] The proportion of imipenem resistant *Acinetobacter* isolates from ICU patients during 1996 and 2007 in Greece ascends from 0% to 85.1%.[27] These latest results were comparatively identical to our rates of resistance.

In conclusion, the results of this study showed that most Acinetobacter isolates were highly resistant to the commonly used antibiotics in the health care settings, including fluoroquinolones, piperacillin + tazobactam, cephalosporins, and imipenem. The findings of this study may have considerable implications for physicians, surgeons, infection control committees, and hospital management personals. The effective implementation of infection control procedures including strict hygiene appears to help control such outbreaks of multidrug-resistant bacteria in hospitals. Moreover, such measures could reduce the rate of infections, duration of hospitalization, and direct and indirect health care costs.

Declaration of Interest: The author(s) declare that they have no conflict of interest to declare

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AUTHORS' CONTRIBUTION

MK: Conceptualization, Write-up, Proofreading, Data collection, Data analysis. AR: Data analysis, Write-up, Proof reading. MH: Data analysis, Write-up, Proof reading. MHR: Conceptualization, Write-up, Proof reading. UW: Data collection, Experimentation, Write-up. MS: Experimentation,

Write-up. MN: Data analysis, Proof reading. MS: Data collection, Data analysis, Proof reading

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