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Ceftolozane-tazobactam, ceftazidime-avibactam and ceftazidime-avibactam plus aztreonam combination: Upcoming hope for hospital-acquired MDR/XDR *Pseudomonas aeruginosa* infections

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ABSTRACT

Background: *Pseudomonas (P. aeruginosa)* is a worrisome multidrug (MDR) or even extensively drug-resistant (XDR) nosocomial pathogen. Ceftolozane/tazobactam(C/T) and ceftazidime/avibactam (CAZ/AVI), novel combinations, were recently approved for the treatment of MDR Gram-negative pathogens. **Objective:** To assess the *in vitro* activity of C/T & CAZ/AVI and against MDR/XDR *P. aeruginosa* isolates, detect the synergistic activity of CAZ/AVI plus aztreonam (ATM) against metallo-β- lactamase**(** MβL**)** producers and to determine the virulence profile of the studied isolates. **Methods:** Eighty *P. aeruginosa* strains were isolated from hospitalized patients and screened for their antimicrobial susceptibility pattern by disk diffusion test. Different resistance mechanisms; beta-lactam hydrolyzing enzymes (ESβLs, AmpC, and class A & B carbapenemases), biofilm production and efflux pump-mediated colistin resistance mechanisms were characterized by the corresponding phenotypic methods. Multiplex PCR verified some resistance (bla_{VIM}, bla_{KPC}, *mcr-1 & mcr-2*) and virulence (*exoU* and *exoS*) genes. We applied E-test strip superposition method to detect synergistic effect between CAZ/AVI and ATM. **Results:** 32.5% and 52.5% of *P. aeruginosa* isolates were recovered as MDR and XDR isolates respectively. The frequency of beta-lactamase production reached 12.5% for ESβLs, 46.25% for AmpC, 21.4% for class A and 55.4% for class B carbapenemases. About 81.3% and 63.7% of the isolates proved susceptibility to CAZ/AVI and C/T respectively. While only 36.3% were ATM susceptible. Interestingly, combined use of CAZ/AVI with ATM completely restored susceptibility among MβL strains. **Conclusion:** Synergistic combination of CAZ/AVI with ATM could be promising therapy against MDR/XDR *P. aeruginosa* infections with MβL production.

Introduction

Multidrug-resistant (MDR) and extensively drug-resistant (XDR) *P. aeruginosa* are challenging nosocomial pathogens that frequently compromise the selection of antimicrobial therapy. The World Health Organization (WHO) listed carbapenem-resistant *P. aeruginosa* as a priority-1 pathogen belonging to difficult-to-treat resistant (DTR) germs with urgent need for new therapeutic strategies to counteract this serious health threat [1].

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Extraordinary capacity to gain antimicrobial resistance via acquired mechanisms is a particular phenomenon amongst *P. aeruginosa* isolates. Such bacterium produces multiple beta-lactamases classified via Ambler class A-D; encompassing extended-spectrum beta-lactamases (ESβLs) and *Klebsiella (pneumoniae) K. pneumoniae* carbapenemases class A (KPCs)[2]. Class B enzymes defined as metallo-b-lactamases (MβLs) can hydrolyze all currently available beta-lactams including; bla_{VIM}, bla_{NDM} & bla_{IMP}. Overproduction of class C AmpC cephalosporinase provides resistance to most beta-lactams as well. Class D beta-lactamases like bla_{OXA-48} affect a wide range of substrates and are not inhibited by traditional beta-lactamase inhibitors [1].

Multiple new agents that displayed *invitro* activity against *P. aeruginosa* (e.g., beta-lactam/beta-lactamase inhibitor combinations and cefiderocol) have been developed and are considered a glimmer of hope for treatment of MDR/XDR *P. aeruginosa* infections [1]. Also, ceftolozane (fifth-generation cephalosporin)-tazobactam(C/T) and ceftazidime-avibactam (CAZ/AVI) have recently granted Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval for treatment of complicated urinary tract, intra-abdominal and hospital-acquired respiratory infections [4].

Ceftolozane-tazobactam proved a potent activity against MDR *P. aeruginosa*; it is not removed by efflux pumps and has a higher affinity with penicillin-binding proteins of cell membrane. Moreover, such combination is stable against ESβLs and overexpressed AmpC [4].

With the advent of novel effective therapeutic lines; ceftazidime-avibactam; third generation cephalosporin β-lactamase inhibitor combination; provided a wide spectrum activity against serine beta-lactamases; however, it is susceptible to hydrolysis by MβLs [5]. On the other hand, the monobactam-aztreonam is stable in the presence of MβLs but can be hydrolyzed by serine beta-lactamases. Thus, the combination of aztreonam with ceftazidime-avibactam is proposed to treat infections caused by MBLs -producing organisms [5].

Among the variety of virulence determinants, type III secretion system (T3SS) is considered as a vital factor responsible for poor clinical outcome of *P. aeruginosa* infections. *ExoS* and *ExoU* proteins of T3SS contribute greatly to pathogenesis of highly virulent strains associated with the severe *P. aeruginosa* drug resistant infections [6].

Our main objectives were to assess the *invitro* activity of ceftolozane-tazobactam, ceftazidime-avibactam and ceftazidime-avibactam plus aztreonam combinations against MDR/XDR *P. aeruginosa* isolates expressing variable resistance mechanisms from patients with hospital-acquired infections (HAIs) at Menoufia University Hospitals (MUHs).

Materials and methods

Specimens' collection

During the period from May 2021 to November 2022, a total of eighty *P. aeruginosa* isolates were obtained from clinical samples of 313 hospitalized patients (210 males and 103 females with a mean age of 44.13 ±18.38 years) who were admitted to different departments and ICUs of MUHs with various clinical forms of hospital associated infections (HAIs) (became evident at least 48 hours after admission) to carry out this analytical study at the Medical Microbiology and Immunology Department, Faculty of Medicine Menoufia University.

Ethical approval

All patients involved in the study gave their informed consent, and the Menoufia University Faculty of Medicine's local ethics committee authorized the study (5/2021MICR10). The research adhered to the principles outlined in the Helsinki Declaration.

Bacterial identification&antibiotic susceptibility testing

A convenient identification of *P. aeruginosa* isolates was achieved based on a scheme described by **Tille** [7] as follows: the isolates were oxidase-positive, triple sugar iron slant gave an alkaline/no change reaction, production of bright bluish (pyocyanin), green (pyoverdin) diffusible pigment on Mueller-Hinton agar and ability to grow at 42°C. Isolates were screened for their antimicrobial susceptibility by way of disk diffusion method according to CLSI guidance [8] except for fosfomycin, where zone diameters were interpreted according to available breakpoints set by the European Committee on Antimicrobial Susceptibility Testing, EUCAST [9]. Colistin (polymyxin E) susceptibility was determined by

agar dilution method for minimal inhibitory concentration (MIC) according to CLSI directions [8]. The obtained isolates were labelled as: MDR (isolate non-susceptible to ≥ 1 agent in ≥ 3 antimicrobial classes) and XDR (isolate non-vulnerable to ≥ 1 agent in all antimicrobial categories however still sensitive to ≤ 2 classes) [1].

Characterization of antimicrobial resistance

Antibiotic resistance mechanisms contributing to the emergence of MDR/XDR *P. aeruginosa* isolates e.g. ESβLs, AmpC, class A & B carbapenemases production, efflux pump as well as biofilm-forming ability were phenotypically assessed as follows:

Detection of ESβLs production

Disk diffusion screening against ceftazidime (30µg), cefepime (30µg) and aztreonam (30µg) was applied on Muller-Hinton agar. Zone diameters less than or equal to 14 mm, 14 mm and 15mm, respectively to at least one disk, have been taken in consideration as potential ESβLs producers [8] ESβLs production was subsequently confirmed by ceftazidime/clavulanic acid combined disk test (CDT). In cases where the inhibition zone around ceftazidime-clavulanic acid discs was at least 5 mm greater than that around the ceftazidime alone, the relevant isolate was interpreted as positive ESβLs producer [10].

Detection of AmpC production

Isolates showing resistance to cefoxitin (inhibition zone<18mm) by disk diffusion method were considered as potential AmpC producers and subsequently confirmed by AmpC disk test [11].

Detection of class A and class B carbapenemases production

Carbapenem-resistant isolates detected by the screening disk diffusion method were subjected to inhibitor- based confirmatory tests by imipenem-boronic acid (BA-CD) [12] and imipenem-EDTA combined disk tests [13] for class A and class B carbapenemases respectively.

Demonstration of biofilm formation by modified Congo red agar method

Congo red agar composed of Congo red dye 0.4 g/L, blood base agar-2 (BAB-2) 40 g/L, glucose 10 g/L and 1000 ml water was prepared. Plates were inoculated and incubated for 48 hrs at 37°C and subsequently 2-4 days at room temperature. Appearance of black colonies was interpreted as positive biofilm- producing strains in contrast to red colonies which were interpreted as negative biofilm producers [14].

Demonstration of efflux pump activity

To determine the role of efflux activity among colistin-resistant MDR/XDR *P. aeruginosa* isolates, colistin was used as a substrate for efflux and carbonyl cyanide 3-chlorophenylhydrazone (CCCP) (Sigma Aldrich; code: C2759) as efflux pump inhibitor. The MIC of colistin in the presence of CCCP is determined and then, compared with the MICs without CCCP [15]. Eight-fold decrease in concentration of colistin MIC in combination with CCCP (10mg/liter) indicates that the resistance mechanism is mediated by efflux pump mechanism [16]

Invitro evaluation of ceftolozane-tazobactam, ceftazidime- avibactam and ceftazidime-avibactam plus aztreonam combinations against MDR/XDR *P. aeruginosa* isolates was determined by the subsequent approach:

i. **Disk diffusion test:** MDR/XDR *P. aeruginosa* isolates with previously characterized resistance and virulence mechanisms were tested against CAZ/AVI (30µg/20µg) and C/T (30µg/10µg) combination disks (MAST, UK) by disk diffusion susceptibility test and zone diameters were interpreted according to CLSI guidelines [8].

ii. **E test strip superposition method:** Initially, MICs of ATM and CAZ/AVI were determined by E test (Liofilchem, Italy) and interpreted according to CLSI breakpoints [8]. To determine the MICs of the ATM plus CAZ/AVI combination, the E test strips of CAZ/AVI were applied on MH agar plates (inoculated with 0.5 McFarland of the test *P. aeruginosa* isolate) for 10 minutes and then removed, and the ATM strip was subsequently deposited on the exact same place. ATM MIC was read, compared to previous readings searching for restoration and enhanced susceptibility to ATM indicating synergy between both drugs [3].

Genotypic characterization of antimicrobial resistance and virulence genes

Multiplex PCR assay was applied for detection of the target genes (bla_{VIM} and bla_{KPC} as genetic determinants of carbapenemases & *mcr-1* and *mcr-2* for colistin resistance) as well as *exoU* and *exoS* among 50 *P. aeruginosa* isolates. The procedure involved DNA extraction with purification using the QIAamp DNA Mini Kit

(Germany) protocol and DNA amplification using thermocycler apparatus (Prime, TECHNE, UK). The amplified products were detected by agarose gel electrophoresis. Sequence of primers for detection of target genes are shown in **table (1).**

Multiplex PCR program used for the amplification of (*exoU, exoS and* bla_{VIM}) genes included; initial denaturation at 95ºC for 2 min and 35 cycles of 95ºC for 1 min, annealing at 60ºC for 45sec then extension at 72ºC for 1min with final extension at 72ºC for 7 min. While (bla_{KPC}, *mcr-1and mcr-2)* genes protocol included initial denaturation at 95ºC for 1min and 40 cycles of 95ºC for 10sec, annealing at 58ºC for 30sec and extension at 72ºC for 1min with final extension step at 72ºC for 10 min

Results

During the period of interest, a total of 313 non-duplicate (one isolate/patient), consecutive isolates were obtained from the study participants of which *P. aeruginosa* (n=80) comprised 25.6% of total isolates. The average age of *P. aeruginosa*-infected patients was 44.13 ±18.38 years, of which 210 (67.1%) were males and 103 (32.9%) were females. About, 22/80 (27.5%) of *P. aeruginosa* isolates were recovered from urine samples, 17 (21.3%) from endotracheal aspirate, 12 (15%) from blood, 11(13.75%) from burn swabs, 9 (11.3%) from pus, surgical drains or wound swabs, 6 (7.5%) from sputum and 3 (3.75%) from ear discharge swabs. Remarkably, ICUs accounted for the highest percentage (41.2%; 33/80) of *P. aeruginosa* isolation. All *P. aeruginosa*-infected patients were subjected to invasive procedures and previously received antibiotics especially colistin and carbapenems by ≥ 43.8% and 40% respectively. About 81.2% of studied patients were hospitalized >7 days and 71% of them had associated comorbidities **(Supplementary data).**

It is noteworthy that *P. aeruginosa* exhibited elevated antimicrobial resistance rates to most of the tested antibiotics with highly reported resistance to gentamicin (75%) followed by ceftazidime and tobramycin (73.8% for each), doripenem (68.7%), meropenem (67.5%), and aztreonam (63.7%). On the contrary, adequate susceptibility was observed for CAZ/AVI, C/T and fosfomycin representing 81.3%, 63.7% and 62.5%, respectively **(Supplementary data)**.

Focusing on various antimicrobial resistance mechanisms using phenotypic screening and confirmatory tests, the current results revealed that ESβLs production was confirmed in 12.5% of *P. aeruginosa* isolates. While, AmpC production was obviously seen among 46.25% using confirmatory disk tests **(Supplementary data, figure 1)**. For carbapenemase detection by inhibitor-based methods, class A and class B metallo-carbapenemase producers were seen among 21.4% and 55.4% of *P. aeruginosa* isolates respectively **(Supplementary data, figure 1)**.

Out of 80 *P. aeruginosa* isolates, 26 (32.5%) and 42 (52.5%) displayed multi- and extensive drug resistance. About 80.8% (21/26) and 76.2% (32/42) of MDR and XDR strains respectively were susceptible to CAZ/AVI. Synchronously, 65.4% and 52.4% of MDR and XDR isolates respectively were susceptible to C/T by disk diffusion screening. Almost 68.5% of C/T - and 90% of CAZ/AVI- resistant XDR *P. aeruginosa* isolates respectively were MβL producers with a statistically significant difference (p value ≤ 0.05) **(Supplementary data)**.

Agar dilution method detected 18.7% (15/80) of *P. aeruginosa* isolates being colistin resistant. The addition of CCCP efflux pump inhibitor decreased MIC of colistin by ≥8 folds in all resistant strains, denoting that all isolates had expressed efflux pump activity. Whereas, 13.3%, 33.3%, 13.3% & 6.7% of these isolates were phenotypically positive for ESβLs, AmpC, class A and B carbapenemase production, respectively. XDR resistance phenotype was observed in 93.3% (14/15) of colistin-resistant isolates **(Supplementary data)**.

Notably, significant biofilm production was seen in approximately 58.7% (47/80) of the processed *P. aeruginosa* isolates using the modified Congo red method, with 81% (34/42) for XDR and 50% (13/26) for MDR strains. About 12.8%, 44.7%, 23.4%, 59.6%, and 23.4% of biofilm-generating isolates expressed ESβL, AmpC, class A, class B, carbapenemase, and efflux pump positive phenotypes respectively. These findings showed statistically significant differences from non-biofilm-producers **(Supplementary data)**.

Multiplex PCR assay showed that bla_{VIM} gene was detected in 16.7% and 19.2% of MDR and XDR isolates, respectively. Meanwhile, bla_{KPC} and *mcr-1* genes were both detected by 3.8% in only XDR isolates. For *exo* virulence genes, *exoU* and *exoS* genes were recovered in 50% and 23.1%

respectively of XDR isolates compared to 16.7% *exoU* and 66.6% *exoS* among MDR ones. None of *P. aeruginosa* isolates under investigation had *mcr-2* gene or showed evidence of *exoU* and *exoS* genes co-existence **(Table 2, Figure 3)**. Genotypic analysis revealed 6.7% detection rate for *mcr-1*, bla_{VIM} & bla_{KPC} genes among colistin-resistant *P. aeruginosa* isolates **(supplementary data)**.

The E test strip superposition method was utilized to evaluate the efficacy of CAZ/AVI in combination with aztreonam against XDR MβLs-producing *P. aeruginosa* isolates. The outcomes demonstrated a synergistic activity proved by restoration of aztreonam susceptibility with aztreonam MICs falling to a range of 3-0.16 µg/ml (The isolate is interpreted as aztreonam susceptible with MICs of less than 8 µg/ml according to CLSI guidelines [8]) **(Supplementary data, Figure 2)**.

Importantly, 72.7%, 22.7%, 4.5% of CAZ/AVI -susceptible *P. aeruginosa* isolates were AmpC, efflux pump, ESβLs producers respectively. None of CAZ/AVI -susceptible isolates were MβLs producers. On the other hand, 83.3% (5/6) and 16.7% (1/6) of CAZ/AVI- resistant *P. aeruginosa* isolates were MβLs and efflux pump producers respectively. None of CAZ/AVI - resistant isolates were AmpC or ESβLs producers **(Table 3)**.

About 73.7%, 21%, 5.3% of C/T -susceptible *P. aeruginosa* isolates were AmpC, efflux pump, ESβLs producers respectively. None of C/T-susceptible isolates were MβLs producers. On the other hand, 55.6%, 22.2% and 22.2% of C/T-resistant *P. aeruginosa* isolates were MβLs, efflux pump and AmpC producers respectively. None of C/T- resistant isolates was ESβLs producer **(Table 4)**

Table 1. Primers used in the study.

Target gene	Sequence (5′ – 3′)	Amplicon Size (bp)	References
exo U	F: 5′-GGGAATACTTTCCGGGAAGTT3′ R: 5′-CGATCTCGCTGCTAATGTGTT-3’	428	[17]
exo S	F: 5′- ATGTCAGCGGGATATCGAAC3′ R: 5′-CAGGCGTACATCCTGTTCCT-3′	230	[18]
mcr-1	F: 5′-CGGTCAGTCCGTTTGTTC-3′ R: 5′-CTTGTTCGGTCTGTAGGG-3′	308	[19]
mcr-2	F: 5′-TGTTGCTTGTGCCGATTGGA-3′ R: 5′AGATGGTATTGTTGGTTGCTG-3′	566	[19]
bla_{VIM}	F: 5′-GATGGTGTTTGGTCGCATA -3′ R: 5′-CGAATGCGCAGCACCAG-3′	390	[20]
bla_{KPC}	F: 5′- GATACCACGTTCCGTCTGG-3′ R: 5′-GCAGGTTCCGGTTTTGTCTC-3′	254	[21]

Table 2. Genotypic profile (blaVIM, blaKPC, mcr-1, mcr-2, exoU& exoS) of MDR and XDR P. aeruginosa isolates by multiplex PCR assay (n=50).

Target genes	MDR *P. aeruginosa* isolates (n=12)		XDR *P. aeruginosa* isolates (n=26)		Non-MDR*P. aeruginosa* isolates (12)		χ^2	p-value
	No.	%	No.	%	No	%		
***blaVIM* (n=7)**	2	16.7	5	19.2	0	0	2.16	0.271
***blaKPC* (n=1)**	0	0	1	3.8	0	0	0.93	0.628
***mcr-1* (n=1)**	0	0	1	3.8	0	0	0.93	0.628
***mcr-2* (n=0)**	0	0	0	0	0	0	-	-
***exoU* (n=15)**	2	16.7	13	50	0	0	11.10	0.004*
***exoS* (n=21)**	8	66.6	6	23.1	7	58.3	8.10	0.017*
Total positive isolates =45								
Total negative isolates =5								
Co-existing genes: Undetectable								

Table 3. In vitro activity of ceftazidime-avibactam by disk diffusion method against MDR and XDR *P. aeruginosa* phenotypes expressing different resistance mechanisms.

Resistance phenotypes of *P. aeruginosa*	**Resistance mechanisms of *P. aeruginosa***	**Ceftazidime/avibactam (Disk diffusion)**				**χ2**	***p*-value**
		S		**R**			
		No	**%**	**No**	**%**		
MDR (n=26)	**Undetectable*= 0**						
	Detectable **=26						
	AmpC	13	61.8	0	0	12.59	0.027*
	AmpC+ efflux	1	4.8	0	0		
	ESβLs + AmpC	3	14.3	1	20		
	ESβLs + MBL	1	4.8	2	40		
	KPC+MBL	2	9.5	0	0		
	MBL+AmpC	1	4.8	2	40		
	Total	21	100	5	100		
XDR (n=42)	**Undetectable*= 8**						
	Detectable* =34						
	AmpC	3	12.5	0	0	22.039	0.005*
	AmpC+ efflux	1	4.2	0	0		
	Efflux	5	20.8	1	10		
	ESβLs	1	4.2	0	0		
	ESβLs + MβLs	2	8.3	0	0		
	KPC + MβLs	9	37.5	1	10		
	MβLs	0	0	5	50		
	MβLs +AmpC	3	12.5	1	10		
	MβLs + efflux	0	0	2	20		
	Total	24	100	10	100		

*Detectable resistance mechanisms *****Undetectable resistance mechanisms χ2: Chi-squared test, *: Statistically significant

Table 4. *In vitro* activity of ceftolozane-tazobactam using disk diffusion method against MDR and XDR *P. aeruginosa* phenotypes expressing different resistance mechanisms.

Resistance phenotypes of *P. aeruginosa*	**Resistance mechanisms of *P. aeruginosa***	**Ceftolozane-tazobactam (Disk diffusion)**				**χ2**	***p*-value**
		S		**R**			
		No	**%**	**No**	**%**		
MDR (n=26)	**Un detectable=0**						
	Detectable =26						
	Total	17	100	9	100	9.32	0.097
	AmpC	11	64.7	2	22.2		
	AmpC + efflux	1	5.9	0	0		
	ESβLs +AmpC	3	17.6	1	11.2		
	ESβLs + MβLs	1	5.9	2	22.2		
	KPC + MβLs	0	0	2	22.2		
	MβLs +AmpC	1	5.9	2	22.2		
XDR (n=42)	**Un detectable =8**						
	Detectable =34						
	Total	15	100	19	100		
	AmpC	3	20	0	0		
	AmpC+ efflux	1	6.7	0	0		
	Efflux	4	26.6	2	10.5		

	ESβLs	1	6.7	0	0	18.857	0.016*
	ESβLs + MβLs	0	0	2	10.5		
	KPC + MβLs	6	40	4	21.1		
	MβLs	0	0	5	26.3		
	MβLs +AmpC	0	0	4	21.1		
	MβLs + efflux	0	0	2	10.5		

χ2: Chi-squared test, *: Statistically significant

Figure 1. A represents positive combined disk confirmatory test for ESβLs production. B represents positive AmpC disk confirmatory test for AmpC production. C represents positive imipenem/EDTA combined disk test for carbapenemase detection. D represents positive imipenem/boronic acid combined disk test for carbapenemase detection.

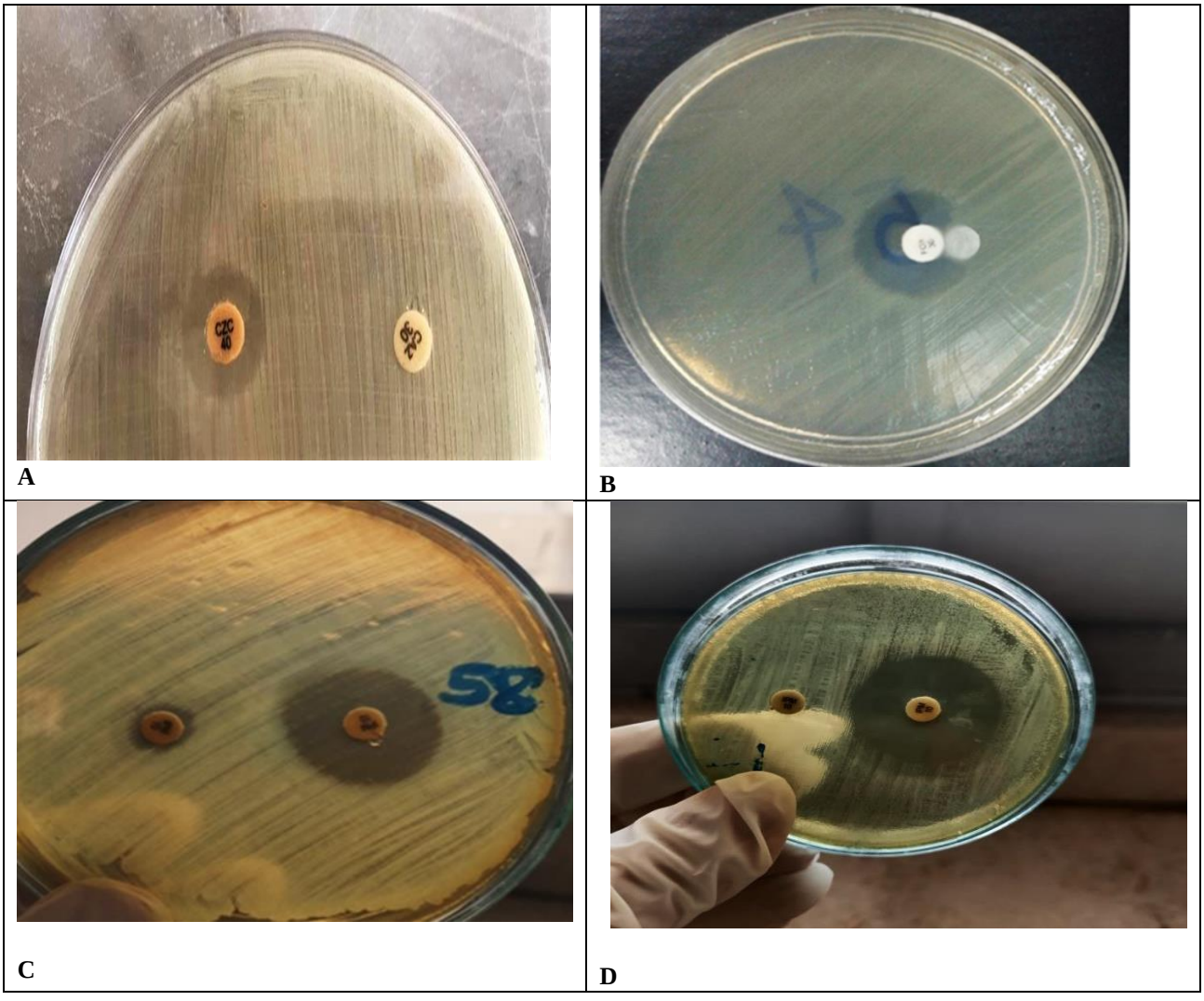


Figure 2. Synergistic effect of aztreonam plus ceftazidime/avibactam combination by superposition method. A: MIC of ceftazidime/avibactam alone was >256 µg/ml B: MIC of aztreonam alone was >256 µg/ml C: MIC of aztreonam plus ceftazidime/avibactam combination became 0.75 µg/ml indicating synergism.

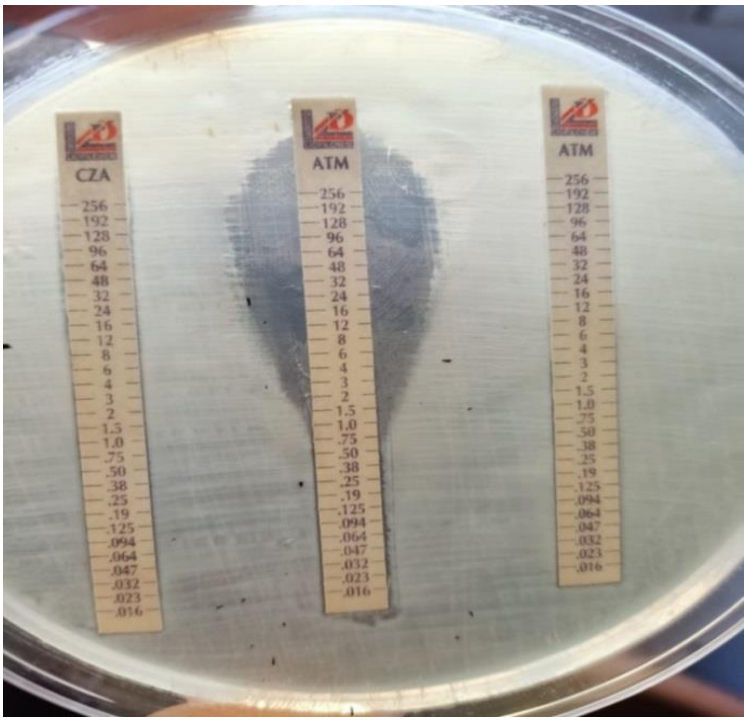


Figure 3a. Agarose gel electrophoresis for the multiplex PCR amplified products of *P. aeruginosa exoU, exoS* and *blaVIM* genes.
Lane M: DNA molecular size marker (100 bp)
Lane 1: represents positive *exoU* (428 bp)
Lanes 3, 4: represent positive *exoS* (230 bp)
Lane 6: represents positive *blaVIM* (390bp)
Lanes 2, 5: represent negative samples.

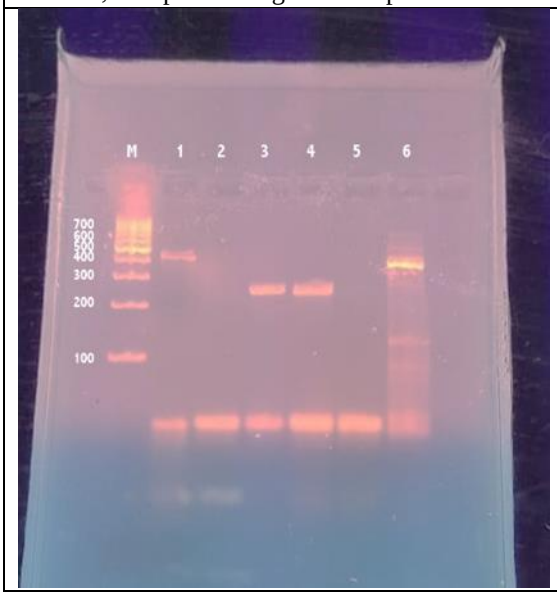
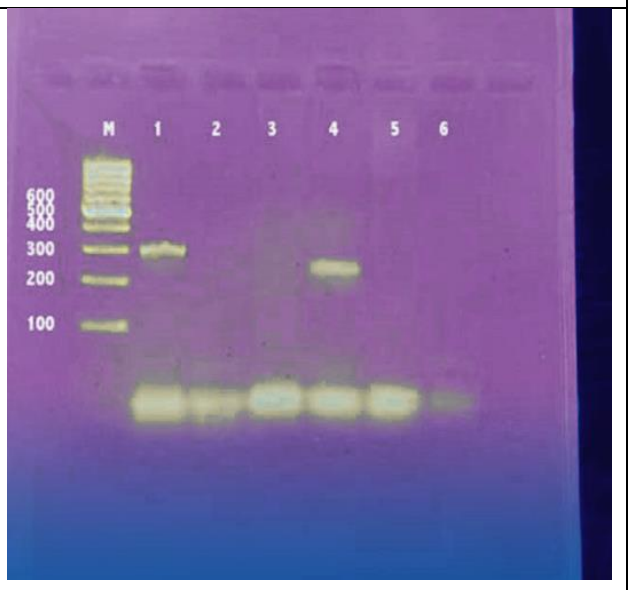


Figure 3 b. Agarose gel electrophoresis for the multiplex PCR amplified products of *P. aeruginosa mcr-1, mcr-2* and *bla KPC* genes.
Lane M: DNA molecular size marker (100 bp)
Lane 1: represents positive *mcr-1* (308 bp)
Lane 4: represents positive *blaKPC* (254bp)
Lanes 2, 3, 5, 6: represent negative samples.



Discussion

P. aeruginosa is a worrisome opportunistic pathogen incriminated in several acute and chronic infections with confusing fatality outcome exceeding 40%. Its reported intrinsic and acquired resistance to available antibiotic options makes prevention and treatment of resultant infections a particularly challenging global concern [22].

During the current study, 80 *P. aeruginosa* strains were isolated from 313 hospitalized patients through 18 months duration where, *P. aeruginosa* accounted for 25.6% of collected isolates. Approximately, similar prevalence rate (22%) was reported in Iran[13], while a higher rate (42.8%) was announced in Egypt [16] in comparison to a lower isolation frequency (4.29%) detected in Nepal [23]. These variances could result from varying antibiotic resistance profiles in addition to regional disparities in the application of infection prevention and control measures

Most isolates were obtained from ICUs (41.2%) and burn unit (13.8%) in accordance with, previous studies [24]. These high rates are possibly due to reduced host defense, impaired immune response, extended hospital stay plus common invasive procedures within ICUs [25]. Regarding specimen distribution, urine and endotracheal aspirate were the most frequent clinical specimens for recovery of *P. aeruginosa* which corresponded with the findings of previous studies [23, 25]

Broad-spectrum antibiotic irrational use in burn ward and ICUs had created selective pressure on bacteria developing multidrug resistance [26]. According to disk diffusion susceptibility results, 32.5% of the screened *P. aeruginosa* isolates were categorized as MDR ones. A previous study [27] in Italy showed a great similarity to ours with 30.2% frequency rate for MDR phenotype. On the other side, only 19% was recorded in Iran [28]. In the same context, our data revealed 52.5% frequency for XDR strains which came in agreement with an Egyptian study [24] which declared 56% of *P. aeruginosa* isolates expressing XDR phenotype possibly justified by antibiotics misuse and genetic changes.

Increasing antimicrobial resistance up to 75% was observed here against aztreonam, meropenem, ceftazidime, tobramycin and gentamycin parallel with other results recorded by various studies [29, 30]. Hopefully, the susceptibility rates reached 81.3%, 63.7% and 62.5% for CAZ/AVI, C/T and fosfomycin respectively; mirroring recent reports [31]. Conversely, the current study found 18.7% colistin resistance among *P. aeruginosa* isolates, which is consistent with **Abd-El Baki *et al.*** [16] explained by colistin abuse in the poultry sector to promote animal growth[16].

Our research aligned with findings from India [10] concerning ESβLs production rate by the combined disk confirmatory test (17.7%) but in contrast to results obtained from KSA [32].The hyper-production of the chromosomal AmpC β-lactamase is the main mechanism driving β-lactam resistance in *P. aeruginosa*. Current results detected AmpC production by 46.25% in agreement with that obtained from Spain [33]. In the same context, class A carbapenemase and class B metallo-beta lactamase producers were detected among 21.4% and 55.4% of isolates respectively which in concordance with various studies [11, 34] with variant antibiotic selection patterns.

In this work, about 58.7% of *P. aeruginosa* isolates were biofilm producers on Congo red agar. Our findings were similar to those reported in Brazil [35] but lower than those recorded in Nepal [36]. Results variability may be affected by type and number of collected samples in each study, nature of infection either acute or chronic and variant phenotypic method used for biofilm detection. Notably, most biofilm- producing isolates here were MDR and XDR by 50% and 81% respectively which came in line with data reported in Iran [28] and Nepal [36].

Interestingly, all colistin- resistant isolates in current work had demonstrated efflux pump activity using CCCP as efflux pump inhibitor in agreement with a research in France [37] where CCCP could reverse colistin resistance for all tested strains *invitro*. About 6.7% and 93.3% of colistin-resistant strains in this study were MDR and XDR, respectively. In Egypt [16], 81.25% of colistin–resistant *P. aeruginosa* isolates were MDR while, only 18.75% of them had efflux pump activity plus XDR phenotype.

Concerning susceptibility pattern of MDR and XDR *P. aeruginosa* isolates to ceftazidime-avibactam and ceftolozane-tazobactam, 80.8% (21/26) and 76.2% (32/42) were susceptible to CAZ/AVI compared to 65.4% and 52.4%, respectively for C/T susceptibility. These findings were matched with studies in USA [38].

Importantly, none of CAZ/AVI - or C/T - susceptible *P. aeruginosa* isolates were MβLs producers. While, more than 72% of them were AmpC producers, an observation parallel to reports in Spain [39].

Although ESβLs production was not reported in this work among both CAZ/AVI and C/T - resistant isolates, more than 83% and 16% of them were MβLs and efflux pump producers. Similarly in recent researches in USA [38], no ESβLs were detected. **Rahimzadeh *et al.*** [40] noticed that 75% of CAZ/AVI and C/T- resistant isolates were MβLs producers. While, **Castanheira *et al.*** [41] observed that, 37% of CAZ/AVI-resistant isolates had efflux pump overexpression. In the study of **Mojica *et al.*** [31], out of 158 C/T- resistant isolates, 43 (27.2%) were MBLs producers, explained probably by different detection methods.

Fortunately, on evaluating the activity of aztreonam plus CAZ/AVI against XDR MβLs-producing *P. aeruginosa* isolates, the aztreonam MIC susceptibility range decreased to 3-0.16 µg/ml denoting restoration of aztreonam susceptibility which indicates the synergistic effect of the combination. Our findings coincided with those of **Lee *et al.*** [42] who documented synergistic combination effect of aztreonam plus CAZ/AVI with restoration of bactericidal activity by 80%. Also, **Emeraud *et al.*** [3] found that, 2 out of 3 aztreonam- resistant *P. aeruginosa* isolates showed synergistic testing of CAZ/AVI and aztreonam with reduced drug MIC by 2 doubling dilutions. In contrast, **Wenzler *et al.*** [43] reported that the combination didn't produce any synergy where additional non- carbapenemase resistance mechanisms like mutations in porins or efflux pumps were reported.

Approximately, 16.7% and 19.2% of MDR and XDR *P. aeruginosa* isolates respectively in our research were positive for bla_{VIM} gene. This came in agreement with studies in Pakistan [44] for MDR isolates and Egypt [45] for XDR ones. Regarding bla_{KPC} gene, it was only detectable among XDR isolates by 3.8% and the same distribution was also observed for *mcr-1* gene. However, none of tested *P. aeruginosa* isolates harbored *mcr-2* gene. Matching results were obtained in Iran [21] and Egypt [16], for the previously mentioned genes respectively.

ExoU and *exoS* type 3 secretion system of *P. aeruginosa* can disrupt the host actin cytoskeleton to interfere with cell-to-cell adhesion, and induce apoptosis of host cells. Concerning detection of these virulence genes in the current study, 50% of XDR *P. aeruginosa* isolates were positive for *exoU* gene compared to only 16.7% for MDR isolates. Regarding the distribution of *exoS* gene, the highest frequency was seen among MDR isolates by 66.6% in comparison to 23% for XDR strains similar to reports from China, Peru and Germany [46].

Conclusion

The increasing resistance of *P. aeruginosa* to available antibiotics is a global ghost. Irrational use of broad-spectrum antibiotic in hospitals and ICUs had dramatically created this ghost. The recently developed antibiotic combinations; ceftazidime/avibactam and ceftolozane/tazobactam are promising treatment options especially against ESβL, AmpC, and class A carbapenemase producers. Aztreonam plus ceftazidime/avibactam combination seems to be an emerging hope against MβLs producers.

Limitation

The inability to perform genotypic analysis to all phenotypically identified.

Declarations

Ethics approval and consent to participate

This analysis was reported to the Local Research Ethical Committee of Faculty of Medicine, Menoufia University (IRB No 5/2021MICR10). Informed consents were obtained from the study participants before involvement in this study.

Consent for publication

Not applicable.

Availability of data and materials

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author contributions

AM and SA designed and contributed to all aspects of the study. AE wrote the manuscript, revised and interpreted the clinical and laboratory data. ME was responsible for samples collection and achieved the practical section of the study. All authors contributed in the revision of the work, edited and approved the final manuscript.

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Conflict of interest

There is no conflict of interest.

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