

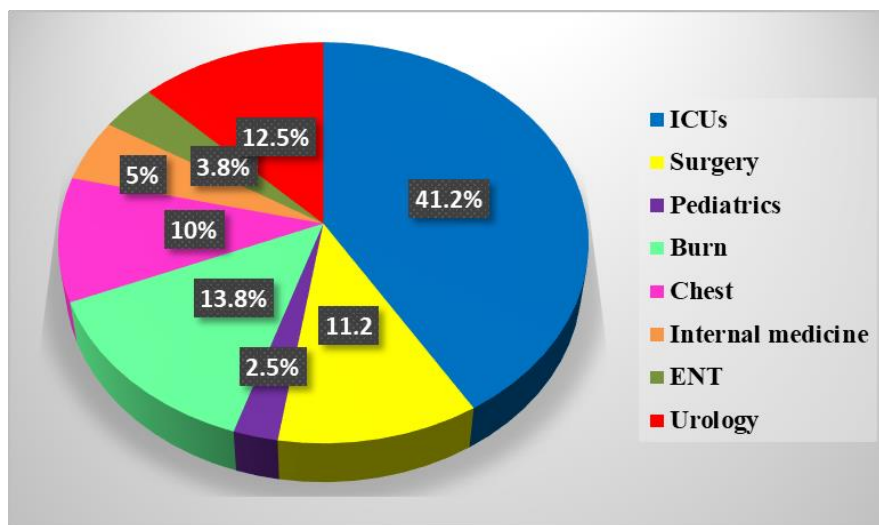


# Microbes and Infectious Diseases

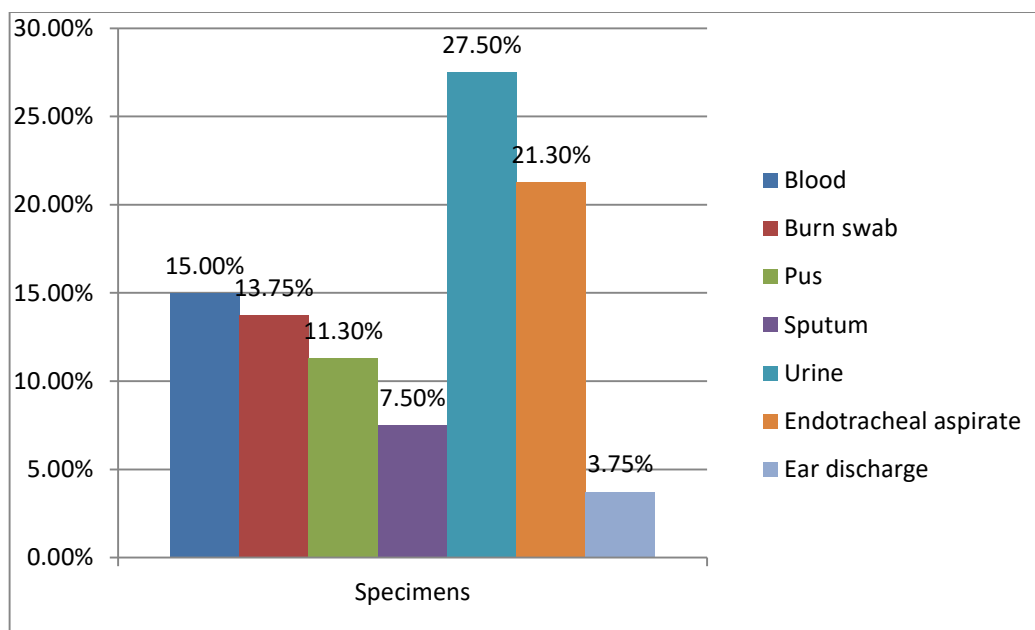
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## Supplementary File

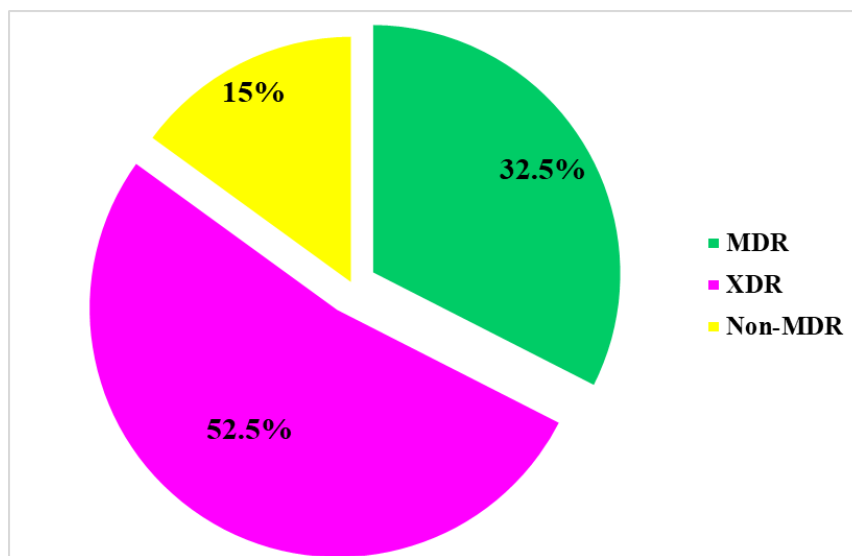
**Figure 4.** Distribution of the obtained *P. aeruginosa* isolates among ICUs and different hospital departments.



**Figure 5.** Distribution of the obtained *P. aeruginosa* isolates among different clinical specimens



**Figure 6.** Antimicrobial resistance phenotypes of the obtained *P. aeruginosa* isolates.



**Figure 7.** Modified Congo Red agar plate (MCRA) for detection of biofilm production among *P. aeruginosa* isolates Letter A: Dry black crystalline colonies i.e. positive for biofilm production. Letter B: Red colored colonies i.e. negative for biofilm production.



**Table 4.** Antimicrobial susceptibility pattern of the obtained *P.aeruginosa* isolates (n=80) by disk diffusion method (CLSI, 2022).

Antimicrobial groups	Antimicrobial agents	Abbrev.	Disk content (µg)	<i>P.aeruginosa</i> isolates (n=80)					
				Interpretive categories					
				S		I		R	
No	%	No	%	No	%				
Penicillins	Piperacillin	PRP	100µg	29	36.3	4	5	47	58.7
B-lactam/β-lactamase inhibitor combinations	Piperacillin-tazobactam	TZP	100/10µg	38	47.5	8	10	34	42.5
	Ceftazidime-avibactam	CZA	30/20 µg	65	81.3	0	0	15	18.7
	Ceftolozane-tazobactam	C/T	30/10 µg	51	63.7	0	0	29	36.3
Cephems	Ceftazidime	CAZ	30µg	21	26.3	0	0	59	73.8
	Cefepime	FEP	30µg	27	33.7	0	0	53	66.3
Monobactams	Aztreonam	ATM	30µg	25	31.3	4	5	51	63.7
Carbapenems	Doripenem	DOR	10µg	25	31.3	0	0	55	68.7
	Imipenem	IPM	10µg	23	28.7	1	1.3	56	70
	Meropenem	MEM	10µg	26	32.5	0	0	54	67.5
Aminoglycosides	Gentamicin	CN	10µg	20	25	0	0	60	75
	Tobramycin	TOB	10µg	21	26.3	0	0	59	73.8
	Amikacin	AK	30µg	30	37.5	4	5	46	57.5
Fluoroquinolones	Ciprofloxacin	CIP	5µg	40	50	0	0	40	50
	Levofloxacin	LEV	5µg	37	46.3	1	1.3	42	52.4
	Norfloxacin	NOR	10µg	47	58.7	0	0	33	41.3
	Ofloxacin	OFX	5µg	45	56.3	0	0	35	43.7
Phosphonic acid	Fosfomycin	FF	50µg	50	62.5	0	0	30	37.5

**Table 5.** Agreement between phenotypic screening and confirmatory methods for ESβLs and AmpC production among *P.aeruginosa* isolates

Phenotypic tests		<i>P. aeruginosa</i> isolates (n=80)			<i>Kappa</i> agreement	p-value
		Combined disk confirmatory test (CDT) for ESβLs		Total		
		+ve (n=10)	-ve (n= 70)			
Disk diffusion screening test	+ve (n=59)	9 (15.3%)	50 (84.7%)	59 (100%)	<i>K</i> 1= 0.060*	<b>p1 &lt;0.001*</b>
	-ve(n= 21)	1 (4.8%)	20 (95.2%)	21 (100%)		
		AmpC disk confirmatory test		Total		
		+ve (n=37)	-ve (n=43)			
Cefoxitin disk diffusion screening method	+ve (n=74)	35 (47.3%)	39 (52.7%)	74 (100%)	<i>K</i> 2= 0.036*	<b>p2 &lt;0.001*</b>
	-ve (n=6)	2 (33.3%)	4 (67.7%)	6 (100%)		

\**K*1: Kappa for ESβLs *K*2: Kappa for AmpC\*: Statistically significant

Kappa interpretation:

Kappa < 0: No agreement

Kappa between 0.00 and 0.20: Slight agreement

Kappa between 0.21 and 0.40: Fair agreement

Kappa between 0.41 and 0.60: Moderate agreement

Kappa between 0.61 and 0.80: Substantial agreement

Kappa between 0.81 and 1.00: Almost perfect agreement.

**Table 6.** Frequency of class A and class B carbapenemases production among *P. aeruginosa* isolates by phenotypic screening and confirmatory methods

Phenotypic tests		<i>P. aeruginosa</i> isolates (n=80)				Total	<i>Kappa</i> agreement	p-value
		Imipenem/boronic acid CD test for class A carbapenemases (KPC)		Imipenem/EDTA CD test for class B carbapenemases (MBL)				
		+ve (n=12)	-ve (n=68)	+ve (n=31)	-ve (n=49)			
Disk diffusion screening test	+ve (n=56)	12 (21.4%)	44 (78.6%)	31(55.4%)	25(44.6%)	56 (100%)	<i>K1</i> = 0.141	<b>p1= 0.013*</b>
	-ve (n=24)	0 (0%)	24 (100%)	0 (0%)	24 (100%)	24 (100%)		

*K1*: Kappa for KPC*K2*: Kappa for MBL

CD: combined disk

p1: p-value for KPC

p2: p-value for MBL

\*: Statistically significant

Kappa interpretation:

Kappa &lt; 0: No agreement

Kappa between 0.00 and 0.20: Slight agreement

Kappa between 0.21 and 0.40: Fair agreement

Kappa between 0.41 and 0.60: Moderate agreement

Kappa between 0.61 and 0.80: Substantial agreement

Kappa between 0.81 and 1.00: Almost perfect agreement.

**Table 7.** Susceptibility pattern of MDR and XDR *P. aeruginosa* isolates to the new beta-lactam/beta-lactamase inhibitor combinations: ceftazidime-avibactam and ceftolozane-tazobactam by disk diffusion method

<i>P. aeruginosa</i>	Ceftazidime-avibactam (Disk diffusion)				Z	p-value	Ceftolozane-tazobactam (Disk diffusion)				Z	p-value
	S		R				S		R			
	No.	%	No.	%			No.	%	No.	%		
<b>MDR isolates (n=26)</b>	21	80.8	5	19.2	4.16	<0.001*	17	65.4	9	34.6	1.94	0.052
<b>XDR isolates (n=42)</b>	32	76.2	10	23.8	3.60	<0.001*	22	52.4	20	47.6	0.22	0.825

\*: Statistically significant

**Table 8.** Correlation between antimicrobial resistance phenotypes and biofilm formation among *P. aeruginosa* isolates

Biofilm formation	MDR <i>P. aeruginosa</i> (n=26)		XDR <i>P. aeruginosa</i> (n=42)		Non-MDR <i>P. aeruginosa</i> (n=12)		Total (n=80)		$\chi^2$	p-value
	No.	%	No.	%	No.	%	No.	%		
<b>Yes (n=47)</b>	13	50	34	81	0	0	47	58.8	28.205	<0.001*
<b>No (n= 33)</b>	13	50	8	19	12	100	33	41.2		

**Table 9. Univariate analysis of different risk factors associated with colistin-resistant *P. aeruginosa* isolates infection**

Characteristics	Colistin-resistant isolates		Colistin– non resistant isolates		$\chi^2$	p-value
	(n=15)		(n=65)			
	No.	%	No.	%		
<b>Hospitalization in ICU</b>	<b>10</b>	<b>66.7</b>	<b>23</b>	<b>35.4</b>	<b>Z= 4.23</b>	<b>&lt;0.001*</b>
<b>**Prolonged hospitalization&gt;21 days</b>	<b>9</b>	<b>60</b>	<b>6</b>	<b>9.2</b>	<b>Z= 4.18</b>	<b>&lt;0.001*</b>
<b>Invasive procedures</b>	<b>15</b>	<b>100</b>	<b>65</b>	<b>100</b>	<b>21.48</b>	<b>&lt;0.001*</b>
<b>Co-morbidities</b>						
• Diabetes mellitus	12	80	45	69.2		
• Hypertension						
• COPD****	0	0	2	3.1		
• Chronic renal failure						
• Chronic liver diseases	0	0	14	21.5		
• Malignancy						
• Combined comorbidities	5	33.3	11	16.9	<b>0.69</b>	<b>0.406</b>
	0	0	0	0		
	0	0	0	0		
	4	26.7	7	10.8		
	3	20	11	16.9		
<b>VAP***</b>	<b>7</b>	<b>46.7</b>	<b>6</b>	<b>9.2</b>	<b>Z=3.550</b>	<b>0.004*</b>
<b>Prior antibiotic exposure</b>	<b>15</b>	<b>100</b>	<b>65</b>	<b>100</b>	<b>Z=1.147</b>	<b>0.250</b>
<b>Prior colistin therapy</b>						
• Monotherapy	15	100	5	7.7		
• Combination therapy						
	0	0	12	18.5	<b>16.94</b>	<b>&lt;0.001*</b>