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Patterns of Antimicrobial Resistance in Clinical Isolates at King Khaled Hospital, Najran, Saudi Arabia: An Analysis of Methicillin-Resistant *Staphylococcus aureus*, Carbapenem Resistance *Enterobacteriaceae*, Extended-Spectrum Beta-Lactamases, and Colistin

Resistance

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Abstract

Antimicrobial resistance (AMR) is a substantial threat in Saudi Arabia. We focus on Methicillin-Resistant *S. aureus* (MRSA), carbapenem-resistant strains, Extended-Spectrum Beta-Lactamases (ESBL) producers, and colistin-resistance. We analyzed 559 samples (blood, urine, wound specimens). ESBLs were the most prevalent (55%), followed by MRSA (22%), combined ESBL and CRE resistance (18%). The ESBL phenotype had a colistin resistance of 71.3%, while ESBL and CRE phenotypes had 22.9%, and CRE had 5.8%. The age group 50+ had 58.7% resistance phenotypes. Urine samples had 49% prevalence of resistant phenotypes, while the highest rate according to ward location was at the OPD (55.8%).

The study highlights critical trends of AMR, emphasizing the urgency to antibiotic regulations, professional development for healthcare professionals, and local studies on AMR for targeted intervention.

Keywords: multidrug-resistant bacteria, extensively drug-resistant bacteria, Enterobacteriaceae, antibiotics, pathogen.

Background

Antibiotic resistance stands as a critical global health issue, leading to increased morbidity, mortality, and healthcare costs. The distribution of antibiotic-resistant pathogens varies across different hospitals, wards, and over time (Hsueh *et al*., 2002). Excessive antibiotic use drives the emergence of antimicrobial resistance, emphasizing the need for rational antibiotic utilization to curb its spread (Prestinaci *et al*., 2015). As more strains become resistant, our ability to combat life-threatening infections diminishes. This phenomenon results from widespread antimicrobial use in humans, animals, and agriculture (Al-Yousef, 2016; Šámal *et al*., 2022). Patients' overuse of antibiotics—whether self-administered or within hospitals—plays a pivotal role in the emergence of resistant strains. Consequently, antimicrobial agents become less effective, prolonging infection duration (Edem *et al*., 2021).

Antibiotic resistance manifests in various categories, including Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Pandrug-Resistant (PDR) strains. Magiorakos *et al*. (2012) defined MDR as resistance to at least one agent in three or more antibiotic classes, XDR as resistance to all but two or fewer classes (with susceptibility in only one or two classes), and PDR as resistance to all agents tested (Magiorakos *et al*., 2012). Notable examples include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamases (ESBLs) (Etok *et al*., 2012; Edem *et al*., 2013). Additionally, carbapenem-resistant Enterobacteriaceae (CRE) and colistin-resistant strains have emerged (Akereuke *et al*., 2023; Devi *et al*., 2024).

In Saudi Arabia, the prevalence of resistant bacterial strains is alarming. *Escherichia coli*, *Klebsiella pneumoniae, Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus* are among the reported culprits (Torumkuney *et al*., 2022). Escalating numbers of these resistant microbes pose a serious threat to global public health in the 21st century (Egwuatu *et al*., 2021). However, there is no report on antimicrobial resistance neither their respective resistance phenotypes in Najran, Saudi Arabia. Therefore, this study, the first of its kind, examines and characterizes antimicrobial resistance among clinical isolates, specifically focusing on MRSA, carbapenem-resistant strains, ESBL producers, and colistin-resistant bacteria at King Khaled Hospital in Najran.

Methods

Study Design and Sample Collection

This study was conducted at King Khalid Hospital, where 559 clinical samples were collected from patients during routine care over a 10-month period in 2023. The samples included urine, pus, high vaginal swabs, nasal swabs, catheter tips, and wound swabs. These samples were obtained from various hospital units, including the Intensive Care Unit (ICU), Outpatient Department (OPD), Female Medical Ward (FMW), Male Medical Ward (MMW), Male Surgical Ward (MSW), and Female Surgical Ward (FSW). Samples were aseptically collected in sterile containers to ensure minimal contamination. Each sample was promptly transported to the laboratory and processed within 4 hours of collection.

Laboratory Analysis and Antibiotic Sensitivity Screening

Samples were subjected to standard microbiological analysis as per Cheesbrough's methodology (2010). Samples were inoculated onto a variety of agar plates, including Blood Agar, Xylose Lysine Deoxycholate (XLD) Agar, Cysteine Lactose Electrolyte Deficient (CLED) Agar, MacConkey Agar, and Chocolate Agar. Most plates were incubated aerobically at 35°C for 24 hours, while Chocolate Agar plates were incubated anaerobically at 37°C for 24 hours. Bacterial identification was performed using the BD-Phoenix 100 automated identification system. Antibiotic susceptibility testing was conducted using the BD-Phoenix 100 system. This automated method allowed for efficient and accurate determination of resistance profiles for the bacterial isolates. The study included all clinical samples collected during routine patient care from participants who provided informed consent. Samples intended for fungal studies were excluded from the analysis.

Ethical Considerations

Ethical approval for the study was obtained from the Medical Research and Ethics Committee of King Khalid Hospital, Saudi Arabia (KACST, KSA: H-11-N-136). Participants were given informed consent forms, and only those who consented were included in the study. All methods were carried out according to relevant guidelines and regulations. This study was conducted by the Declaration of Helsinki.

Results

Percentage Occurrences of Bacterial Isolates in Clinical Samples

Figure 1 shows the percentage of various bacterial isolates found in different clinical samples. *E. coli* (51%) was the most prevalent isolate; followed by *S. aureus* (22%), *Klebsiella pneumoniae* (14%), *P. aeruginosa* (6%), *Acinetobacter baumannii* (6%), while *Enterococcus faecium* (1%) was the least prevalent (Figure 1).

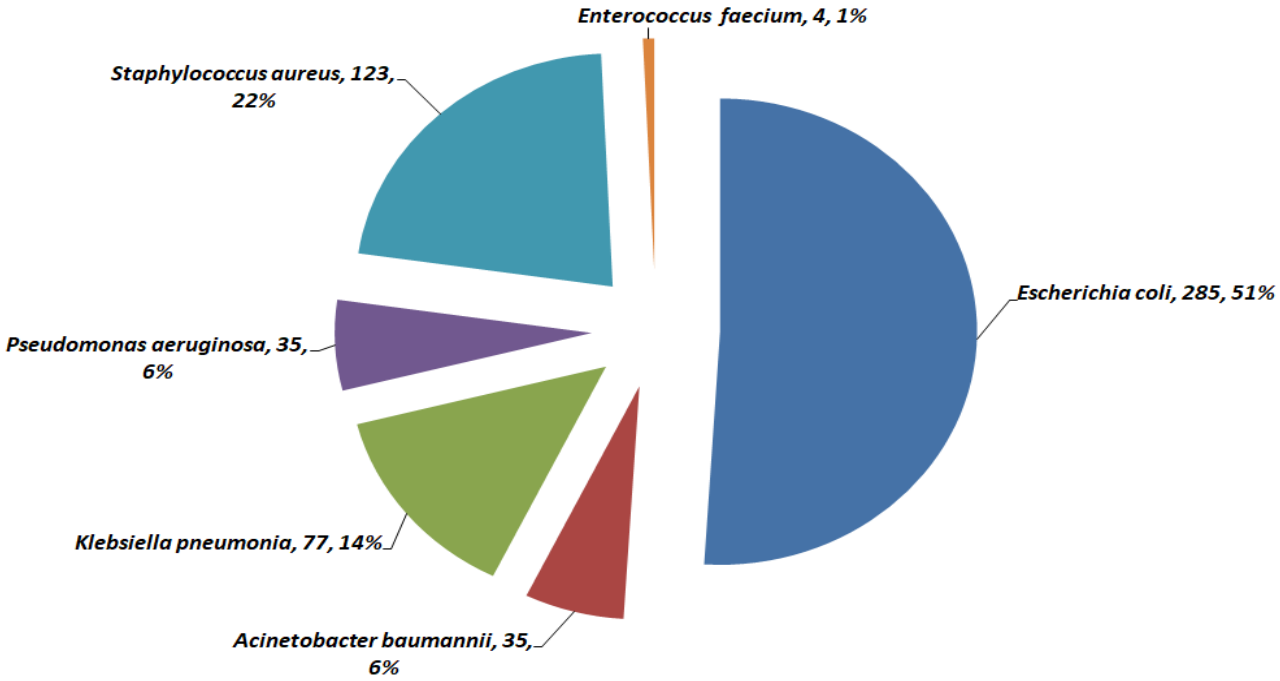

Figure 1: Prevalence of Bacterial Isolates

Resistance Profile of MRSA and VRE

Table 1 presents the resistance profile of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Vancomycin-Resistant *Enterococcus faecium* (VRE) against various antibiotics. For MRSA (n=123), 21.9% of the strains were resistant to clindamycin, 100% were resistant to cefotaxime, cefoxitin, and oxacillin, 0.8% were resistant to ceftaroline, and 36.6% were resistant to erythromycin. None of the MRSA strains were resistant to daptomycin, linezolid, trimeth-sulfamethoxazole, or vancomycin. Additionally, 13% of the MRSA strains were resistant to gentamicin, 20.3% to levofloxacin, and 15.4% to tetracycline (Table 1). For VRE (n=4), 100% of the strains were resistant to clindamycin, cefotaxime, cefoxitin, ceftaroline, erythromycin, gentamicin, levofloxacin, tetracycline, and vancomycin, while none were resistant to daptomycin, linezolid, or trimeth-sulfamethoxazole (Table 1).

Table 1: Resistance profile of MRSA and VRE

Antibiotics	**MRSA** *Staphylococcus aureus* **(123)**	**VRE** *Enterococcus faecium* **(4)**
Clindamycin	27(21.9)	4 (100)
Cefotaxime	123 (100)	4 (100)
Cefoxitin	123 (100)	4 (100)

Ceftaroline	1 (0.8)	4 (100)
Daptomycin	0	0
Erythromycin	45 (36.6)	4 (100)
Gentamicin	16 (13)	4 (100)
Levofloxacin	25 (20.3)	4 (100)
Linezolid	0	0
Oxacillin	123 (100)	-
Tetracyclin	19 (15.4)	1 (25)
Trimeth-Sulfamethoxazole	0	0
Vancomycin	0	4 (100)

methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE)

Resistance profile of ESBL, CRE, and combined ESBL + CRE

Table 2 outlines the resistance profile of Extended-Spectrum Beta-Lactamase (ESBL) and Carbapenem-Resistant Enterobacteriaceae (CRE) against various antibiotics. For *Escherichia coli* (n=276 for ESBL, n=9 for CRE), strains producing either phenotype had high resistance to ampicillin, cefepime, ceftazidime, ceftriaxone, cefuroxime, colistin, and levofloxacin, but exhibited low or no resistance to amikacin, tigecycline, imipenem, meropenem and Piperacillin-Tazobactam (Table 2). For *Klebsiella pneumoniae* (n=32 for ESBL, n=16 for CRE), strains producing either phenotype demonstrated high resistance to ampicillin, cefepime, ceftriaxone, cefuroxime, imipenem, meropenem, colistin, and levofloxacin, but low or no resistance to amikacin, tigecycline, and Piperacillin-Tazobactam (Table 2). For isolates that produced both ESBL and CRE phenotypes; *Pseudomonas aeruginosa* (n=35), *Acinetobacter baumannii* (n=35) and *Klebsiella pneumoniae* (n=29), all exhibited very high resistance to all antibiotics except Piperacillin-Tazobactam, Tigecycline and Trimethoprim-Sulfamethoxazole which demonstrated low resistance (Table 2).

Table 2: Resistance profile of ESBL, CRE, ESBL + CRE

	ESBL		**CRE**		**ESBL + CRE**		
Antibiotics	***Escherichia coli* (276)**	***Klebsiella pneumoniae* (32)**	***Escherichia coli* (9)**	***Klebsiella pneumoniae* (16)**	***Pseudomonas aeruginosa* (35)**	***Klebsiella pneumoniae* (29)**	***Acinetobacter baumannii* (35)**
Amikacin	0	22 (68.8)	0	7 (43.8)	35 (100)	23 (79.3)	28 (80)
Amoxicillin-Clavulanate	124 (44.9)	26 (81.3)	1 (11.1)	9 (56.3)	35 (100)	29 (100)	35 (100)
Ampicillin	275 (99.7)	32 (100)	9 (100)	16 (100)	35 (100)	29 (100)	35 (100)
Cefepime	276 (100)	32 (100)	0 (0)	0 (0)	34 (97.1)	29 (100)	33 (94.3)
Ceftazidime	276 (100)	30 (81.3)	6 (66.7)	9 (56.3)	35 (100)	29 (100)	35 (100)
Ceftriaxone	276 (100)	32 (100)	0 (0)	1 (6.3)	35 (100)	29 (100)	35 (100)
Cefuroxime	276 (100)	32 (100)	0 (0)	0 (0)	35 (100)	29 (100)	35 (100)
Colistin	276 (100)	32 (100)	9 (100)	16 (100)	35 (100)	29 (100)	35 (100)
Imipenem	1 (0.4)	0	9 (100)	15 (93.7)	34 (97.1)	27 (93.1)	35 (100)
Levofloxacin	255 (92.4)	32 (100)	9 (100)	16 (100)	35 (100)	29 (100)	35 (100)
Meropenem	3 (1.1)	0	9 (100)	16 (100)	35 (100)	29 (100)	35 (100)
Piperacillin-Tazobactam	19 (6.9)	4 (12.5)	8 (88.9)	8 (50)	18 (51.4)	23 (79.3)	35 (100)
Tigecycline	1 (1.1)	2 (6.3)	0	1 (6.3)	11 (31.4)	6 (20.7)	5 (14.3)
Trimethoprim-Sulfamethoxazole	159 (57.6)	21 (65.6)	8 (88.9)	16 (100)	32 (91.4)	27 (93.1)	33 (94.3)

Extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE)

Prevalence of Resistance Phenotype

Among the 559 isolates studied, the prevalence of drug-resistant phenotypes varied significantly, and this is shown in Table 3. ESBL-producing organisms exhibited the highest prevalence (55%),

followed by MRSA resistance (22%), combined ESBL and CRE resistance (18%), CRE resistance alone (5%), and the least common occurrence (1%) of VRE-producing organisms (Table 3). Notably, *Escherichia coli* showed a 49.4% ESBL production rate and a 1.6% CRE production rate, while *Klebsiella pneumoniae* demonstrated 5.7% ESBL, 2.9% CRE, and 5.2% combined ESBL and CRE resistance (Table 3). *Pseudomonas aeruginosa* and *Acinetobacter baumannii* each had 6.3% of isolates with combined ESBL and CRE resistance (Table 3). *Staphylococcus aureus* had a 22% prevalence of MRSA, and *Enterococcus faecium* exhibited a 1% rate of vancomycin resistance (Table 3).

Table 3: Prevalence of Resistance phenotypes across Bacterial Isolates

Organisms	ESBL	CRE	ESBL + CRE	MRSA	VRE
Escherichia coli	276 (49.4)	9 (1.6)	0	0	0
Klebsiella pneumoniae	32 (5.7)	16 (2.9)	29 (5.2)	0	0
Acinetobacter baumannii	0	0	35 (6.3)	0	0
Pseudomonas aeruginosa	0	0	35 (6.3)	0	0
Staphylococcus aureus	0	0	0	123 (22)	0
Enterococcus faecium	0	0	0	0	4 (1)
Total	**308 (55)**	**25 (5)**	**99 (18)**	**123 (22)**	**4 (1)**

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE).

Prevalence of colistin resistance

Colistin resistance rates varied significantly among different resistance phenotypes, as shown in Table 4. ESBL phenotype isolates had a high resistance rate of 71.3%, while those with both ESBL and CRE phenotypes showed a lower rate of 22.9% (Table 4). The CRE phenotype alone had the lowest resistance rate at 5.8%. Among bacterial species, *Escherichia coli* and *Klebsiella pneumoniae* with the ESBL phenotype had colistin resistance rates of 96.8% and 41.6%, respectively (Table 4). Notably, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates with both ESBL and CRE phenotypes displayed 100% colistin resistance (Table 4).

Table 4: Prevalence of colistin resistance

Organisms	ESBL	CRE	ESBL + CRE
Escherichia coli	276 (96.8)	9 (3.3)	-
Klebsiella pneumoniae	32 (41.6)	16 (20.8)	29 (37.7)
Acinetobacter baumannii	-	-	35 (100)
Pseudomonas aeruginosa	-	-	35 (100)
Total	**308 (71.3)**	**25 (5.8)**	**99 (22.9)**

Extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE)

Distribution of Resistance Phenotypes According to Age and Gender

Table 4 outlines the distribution of antibiotic resistance phenotypes among various bacterial organisms, categorized by age groups and gender. Of the 559 participants, age group 50+ had the highest prevalence of resistance phenotypes with 58.7%, and the least was ≤19 age group with 4.3% (Table 5). The distribution of antibiotic resistance phenotypes across various bacterial organisms revealed that *E. coli* with ESBL + Colistin phenotype had the highest prevalence in the 50+ age group at 64.9%, and a higher prevalence in females at 53.3% (Table 5). Similarly, *E. coli* with CRE + Colistin phenotype was most prevalent in the 50+ age group at 77.8%, with females

showing an 88.9% prevalence (Table 5). *Acinetobacter baumannii* with ESBL + CRE + Colistin phenotype had the highest prevalence of 42.9% for the 50+ age group, with male gender having highest prevalence at 82.9% (Table 5). *Klebsiella pneumoniae* showed a high prevalence in the 50+ age group across all phenotypes: CRE + Colistin at 68.8%, ESBL + Colistin at 78.1%, and ESBL + CRE + Colistin at 75.9%, with males having the highest prevalence for the latter two phenotypes at 53.1% and 62.1%, respectively (Table 5). *Pseudomonas aeruginosa* with ESBL + CRE + Colistin phenotype had a 77.1% prevalence in the 50+ age group, with 80% of cases being male. *Staphylococcus aureus* with the MRSA phenotype had a 32.5% prevalence in the 50+ age group and a 70.7% prevalence in males (Table 5). Lastly, *Enterococcus faecium* with the VRE phenotype was most prevalent in the 50+ age group at 66.7%, with a 75% prevalence in males (Table 5).

Table 5: Distribution of Resistance phenotypes according to age and Gender

Organism	Resistance phenotype	Age					Gender	
		≤19	**20-29**	**30-39**	**40-49**	**50+**	**Male**	**Female**
Escherichia coli	ESBL + Colistin (276)	7 (2.5)	24 (8.7)	39 (14.1)	27 (9.8)	179 (64.9)	129 (46.7)	147 (53.3)
	CRE + Colistin (9)	0	1 (11.1)	1 (11.1)	0	7 (77.8)	1 (11.1)	8 (88.9)
Acinetobacter baumannii	ESBL + CRE + Colistin (35)	2 (5.7)	8 (22.9)	6 (17.1)	4 (11.4)	15 (42.9)	29 (82.9)	6 (17.1)
	CRE + Colistin (16)	0	1 (6.3)	3 (18.8)	1 (6.3)	11 (68.8)	7 (43.8)	9 (56.3)
Klebsiella pneumoniae	ESBL + Colistin (32)	0	1 (3.1)	3 (9.4)	3 (9.4)	25 (78.1)	17 (53.1)	15 (46.9)
	ESBL + CRE + Colistin (29)	1 (3.4)	2 (6.9)	3 (10.3)	1 (3.4)	22 (75.9)	18 (62.1)	11 (37.9)
Pseudomonas aeruginosa	ESBL + CRE + Colistin (35)	1 (2.9)	2 (5.7)	2 (5.7)	3 (8.6)	27 (77.1)	28 (80)	7 (20)
Staphylococcus aureus	MRSA (123)	12 (9.8)	25 (20.3)	25 (20.3)	21 (17.1)	40 (32.5)	87 (70.7)	36 (29.3)
Enterococcus faecium	VRE (4)	1 (33.3)	1 (33.3)	0	0	2 (66.7)	3 (75)	1 (25)
	Total	**24 (4.3)**	**65 (11.6)**	**82 (14.7)**	**60 (10.7)**	**328 (58.7)**	**319 (57.1)**	**240 (42.9)**

Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE)

Distribution of Resistance Phenotypes According to Ward Location

Table 5 details the distribution of antibiotic resistance phenotypes among various bacterial organisms, categorized by ward location. The prevalence rates according to ward location were highest in the OPD at 55.8%, followed by 14.7% in the ICU, 12.9% in the MSW, 9.3% in the MMW, 3.9% in the FSW, and lowest in the FMW at 3.4% (Table 6). The distribution of resistance phenotypes according to ward location revealed that in the OPD, *Escherichia coli* (ESBL + Colistin) had the highest prevalence at 65.9%, while in the ICU, *Escherichia coli* (CRE + Colistin) was most common at 55.6%. *Acinetobacter baumannii* (ESBL + CRE + Colistin) also showed high prevalence in the ICU, accounting for 54.3% (Table 6). Among *Klebsiella pneumoniae* isolates, the CRE + Colistin phenotype predominated in the ICU (43.8%), whereas the ESBL + Colistin phenotype peaked in the OPD (68.8%). Notably, the ESBL + CRE + Colistin phenotype for *Klebsiella pneumoniae* was prevalent in both the OPD and ICU, with a combined prevalence

of 37.9%. *Pseudomonas aeruginosa* (ESBL + CRE + Colistin) exhibited the highest prevalence in the ICU (34.3%) (Table 6). *Staphylococcus aureus* (MRSA) was most found in the OPD (62.6%), while *Enterococcus faecium* (VRE) was predominant in the ICU (50%) (Table 6).

Table 6: Distribution of resistance phenotypes according to ward location

Organism	Resistance Phenotype	Ward					
		OPD	**MMW**	**MSW**	**FMW**	**FSW**	**ICU**
Escherichia coli	ESBL + Colistin (276)	182 (65.9)	27 (9.8)	24 (8.7)	11 (4)	10 (3.6)	22 (8)
	CRE + Colistin (9)	0	4 (44.4)	0	0	0	5 (55.6)
Acinetobacter baumannii	ESBL + CRE + Colistin (35)	6 (17.1)	4 (11.4)	3 (8.6)	2 (5.7)	1 (2.9)	19 (54.3)
Klebsiella pneumoniae	CRE + Colistin (16)	5 (31.3)	2 (12.5)	0	0	2 (12.5)	7 (43.8)
	ESBL + Colistin (32)	22 (68.8)	2 (6.3)	7 (21.9)	0	1 (3.1)	0
	ESBL + CRE + Colistin (29)	11 (37.9)	0	4 (13.8)	1 (3.4)	2 (6.9)	11 (37.9)
Pseudomonas aeruginosa	ESBL + CRE + Colistin (35)	8 (22.9)	4 (11.4)	9 (25.7)	0	2 (5.7)	12 (34.3)
Staphylococcus aureus	MRSA (123)	77 (62.6)	9 (7.3)	25 (20.3)	4 (3.3)	4 (3.3)	4 (3.3)
Enterococcus faecium	VRE (4)	1 (25)	0	0	1 (25)	0	2 (50)
	Total	**312 (55.8)**	**52 (9.3)**	**72 (12.9)**	**19 (3.4)**	**22 (3.9)**	**82 (14.7)**

Intensive Care Unit (ICU), Outpatient Department (OPD), Female Medical Ward (FMW), Male Medical Ward (MMW), Male Surgical Ward (MSW), and Female Surgical Ward (FSW), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE)

Distribution of Resistance Phenotypes According to Sample Type

Table 6 provides a comprehensive breakdown of antibiotic resistance phenotypes among different bacterial organisms, categorized by sample type. Urine sample had the highest prevalence of 49%, followed by wound samples with 22.7%, pus sample with 18.6%, nasal swabs with 7.3%, catheter tip with 1.3% and least was HVS with 1.1% (Table 7). *Escherichia coli* (ESBL + Colistin) had the highest prevalence in urine samples (68.5%), while *Escherichia coli* (CRE + Colistin) showed equal prevalence in pus and urine samples (44.4% each). *Acinetobacter baumannii* (ESBL + CRE + Colistin) was most common in wound samples (48.6%) (Table 7). Among *Klebsiella pneumoniae*, the CRE + Colistin phenotype had the highest prevalence in urine samples (68.8%), while the ESBL + Colistin phenotype was most prevalent in urine (75%). The ESBL + CRE + Colistin phenotype for *Klebsiella pneumoniae* showed the highest prevalence in urine samples (51.7%). *Pseudomonas aeruginosa* (ESBL + CRE + Colistin) had the highest prevalence in urine samples (40%), and *Staphylococcus aureus* (MRSA) was most prevalent in wound samples (42.3%). Finally, *Enterococcus faecium* (VRE) had the highest prevalence in urine samples (75%) (Table 7).

Table 7: Distribution of resistance phenotypes according to sample type

Organism	Resistance Phenotype	Sample					
		Wound	**Pus**	**Urine**	**Catheter Tip**	**Nasal Swab**	**HVS**
Escherichia coli	ESBL + Colistin (276)	34 (12.3)	30 (10.9)	189 (68.5)	2 (0.7)	21 (7.6)	0
	CRE + Colistin (9)	0	4 (44.4)	4 (44.4)	0	1 (11.1)	0

Acinetobacter baumannii	ESBL + CRE + Colistin (35)	17 (48.6)	6 (17.1)	10 (28.6)	0	1 (2.9)	1 (2.9)
	CRE + Colistin (16)	3 (18.8)	1 (6.3)	11 (68.8)	0	1 (6.3)	0
Klebsiella pneumoniae	ESBL + Colistin (32)	3 (9.4)	4 (12.5)	24 (75)	0	1 (3.1)	0
	ESBL + CRE + Colistin (29)	4 (13.8)	6 (20.7)	15 (51.7)	1 (3.4)	2 (6.9)	1 (3.4)
Pseudomonas aeruginosa	ESBL + CRE + Colistin (35)	13 (37.1)	4 (11.4)	14 (40)	1 (2.9)	3 (8.8)	0
Staphylococcus aureus	MRSA (123)	52 (42.3)	49 (39.8)	4 (3.3)	3 (2.4)	11 (8.9)	4 (3.3)
Enterococcus faecium	VRE (4)	1 (25)	0	3 (75)	0	0	0
	Total	**127 (22.7)**	**104 (18.6)**	**274 (49.0)**	**7 (1.3)**	**41 (7.3)**	**6 (1.1)**

methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE)

Distribution of Resistance Phenotypes According to Gender against Ward Location

Table 7 describes the distribution of resistance phenotypes according to gender against ward location. In our study involving 559 participants, we observed varying prevalence rates of antibiotic resistance phenotypes among different bacterial organisms. Males had the highest prevalence in the OPD (27.2%), while females had the highest in the same setting (28.6%) (Table 8). Specifically, *Escherichia coli* (ESBL + Colistin) showed the highest prevalence in males within the OPD at 56.6%, while females had the highest prevalence in the same setting at 74.1% (Table 8). For *Escherichia coli* (CRE + Colistin), males had a 100% prevalence in MMW, whereas females showed the highest prevalence (62.5%) in ICU (Table 8). Among *Acinetobacter baumannii* (ESBL + CRE + Colistin), males had the highest prevalence (55.2%) in the ICU, while females also had their highest prevalence (50%) in the same ward (Table 8). Moving to *Klebsiella pneumoniae*, males exhibited the highest prevalence (57.1%) in the ICU for the CRE + Colistin phenotype, whereas females had the highest prevalence (33.3%) in the OPD. For the ESBL + Colistin phenotype in *Klebsiella pneumoniae*, males dominated in the OPD (47.1%), while females had an even higher prevalence (93.3%) in the same setting. The ESBL + CRE + Colistin phenotype for *Klebsiella pneumoniae* was most prevalent in males (50%) in the ICU and in females (54.5%) in the OPD (Table 8). Among *Pseudomonas aeruginosa* (ESBL + CRE + Colistin), males had the highest prevalence (35.7%) in the ICU, while females showed a slightly lower prevalence (28.6%) in the same ward. *Staphylococcus aureus* (MRSA) had males with the highest prevalence (59.8%) in the OPD and females with the highest prevalence (69.4%) in the same setting. Finally, *Enterococcus faecium* (VRE) was most prevalent in males (66.7%) in the ICU and in females (100%) in the Female Medical Ward (FMW) (Table 8).

Mean and Standard Deviation Description (for Table 4)

In Table 4 the mean values represent the average number of resistance cases in each ward for both male and female patients, while the standard deviation indicates the variability or dispersion of these cases. For example, in the ICU, male patients had an average (mean) of 2.12 resistance cases with a standard deviation of 1.657, indicating a moderate spread around the mean value.

Table 8: Distribution of Resistance Phenotypes According to Gender against Ward location

Organism	**Resistance Phenotype**	**Gender**	**Ward**						**Mean**	**Std. Deviation**
			OPD	**MMW**	**MSW**	**FMW**	**FSW**	**ICU**		
Escherichia coli	ESBL + Colistin	Male (129)	73 (56.6)	16 (12.4)	24 (18.6)	0	0	16 (12.4)	2.12	1.657
		Female (147)	109 (74.1)	11 (7.5)	0	11 (7.5)	10 (6.8)	6 (4.1)	1.78	1.507

	CRE + Colistin	Male (1)	0	1 (100)	0	0	0	0	2.00	.
		Female (8)	0	3 (37.5)	0	0	0	5 (62.5)	4.50	2.070
Acinetobacter baumannii	ESBL + CRE + Colistin	Male (29)	5 (17.2)	3 (10.3)	3 (10.3)	2 (10.3)	0	16 (55.2)	4.28	2.086
		Female (6)	1 (16.7)	1 (16.7)	0	0	1 (16.7)	3 (50)	4.33	2.251
Klebsiella pneumoniae	CRE + Colistin	Male (7)	2 (28.6)	1 (14.3)	0	0	0	4 (57.1)	4.00	2.517
		Female (9)	3 (33.3)	1 (11.1)	0	0	2 (22.2)	3 (33.3)	3.67	2.345
	ESBL + Colistin	Male (17)	8 (47.1)	2 (11.8)	7 (41.2)	0	0	0	1.94	.966
		Female (15)	14 (93.3)	0	0	0	1 (6.7)	0	1.27	1.033
	ESBL + CRE + Colistin	Male (18)	5 (27.8)	0	4 (22.2)	0	0	9 (50)	1.94	.966
		Female (11)	6 (54.5)	0	0	1 (9.1)	2 (18.2)	2 (18.2)	1.27	1.033
Pseudomonas aeruginosa	ESBL + CRE + Colistin	Male (28)	6 (21.4)	3 (10.7)	9 (32.1)	0	0	10 (35,7)	3.54	2.009
		Female (7)	2 (28.6)	1 (14.3)	0	0	2 (28.6)	2 (28.6)	3.71	2.289
Staphylococcus aureus	MRSA	Male (87)	52 (59.8)	9 (10.3)	25 (28.7)	0	0	1 (1.1)	1.74	1.005
		Female (36)	25 (69.4)	0	0	4	4 (11.1)	3 (8.3)	2.19	1.880
Enterococcus faecium	VRE	Male (3)	1 (33.3)	0	0	0	0	2 (66.7)	4.33	2.887
		Female (1)	0	0	0	1 (100)	0	0	4.00	.
	Total	**Male**	**152 (27.2)**	**35 (6.3)**	**72 (12.9)**	**2 (0.4)**	**0**	**58 (10.4)**		
		Female	**160 (28.6)**	**17 (3.0)**	**0**	**17 (3.0)**	**22 (3.9)**	**24 (4.3)**		

Intensive Care Unit (ICU), Outpatient Department (OPD), Female Medical Ward (FMW), Male Medical Ward (MMW), Male Surgical Ward (MSW), and Female Surgical Ward (FSW), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and extended-spectrum beta-lactamases (ESBLs) carbapenem-resistant Enterobacteriaceae (CRE)

Discussion

Antimicrobial resistance (AMR) is a critical challenge in Saudi Arabia, driven by the high prevalence of extended-spectrum beta-lactamase (ESBL) and carbapenem-resistant Enterobacteriaceae (CRE) phenotypes. The widespread resistance to commonly used antibiotics, even last resort options, underscores the urgency of implementing stricter antibiotic stewardship programs and infection control measures. The study conducted at King Khaled Hospital in Najran, Saudi Arabia, highlights concerning trends in antimicrobial resistance (AMR) among clinical isolates, underscoring the urgent need for improved antibiotic stewardship and stringent infection control measures. *E. coli* emerged as the most prevalent bacterial isolate, accounting for 51% of

cases. This aligns with findings from studies by Mutair *et al*. (2021) in Saudi Arabia and Akinjogunla *et al*. (2024) in Nigeria, where *E. coli* was also the predominant isolate at rates of 42.2% and 32.3%, respectively. *E. coli*'s high prevalence is attributable to its common role in urinary tract infections (UTIs) and surgical site infections, prevalent in both community and hospital settings. The large proportion of urine samples submitted for microbiological analysis makes it expected for *E. coli* to frequently emerge as the most common isolate. The spread of *E. coli* is facilitated within healthcare facilities by patient movement, contaminated surfaces, and inadequate hygiene, persisting in environments such as sinks, drains, and medical equipment. Additionally, *E. coli* can transfer resistance genes from animals to humans, with antibiotic use in livestock and contaminated food contributing to its prevalence (Regassa *et al*., 2023).

Staphylococcus aureus was also significant due to its role in skin and soft tissue infections, surgical site infections, and bloodstream infections. The hospital environment provides ample opportunities for *S. aureus* transmission. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* are prevalent in nosocomial infections, particularly in intensive care units (ICUs), where they are associated with severe infections such as pneumonia, bloodstream infections, and wound infections. These pathogens' ability to survive in hospital environments and develop resistance to multiple antibiotics contribute to their persistence. The low prevalence of *Enterococcus faecium* (1%) might be due to its specific association with certain hospital-acquired infections, particularly in immunocompromised patients or those with prolonged hospital stays. Despite its lower prevalence, the emergence of vancomycin-resistant Enterococcus (VRE) strains makes it a significant concern for infection control and patient management.

The study revealed that 55% of all isolates were ESBL-producing organisms concerning statistics linked to the widespread use of broad-spectrum cephalosporins (Miteu *et al*., 2023). These bacteria develop resistance by producing beta-lactamase enzymes that break down beta-lactam antibiotics (Sarkar *et al*., 2019). Conversely, CRE-producing isolates were less prevalent. This trend likely results from the reserved use of carbapenems for severe infections, aiming to prevent widespread resistance, as these antibiotics are considered last-resort options (Rousham *et al*., 2018). A retrospective study in the central region of Saudi Arabia identified 37.1% and 27.8% of *E. coli* and Klebsiella species isolates as ESBL, respectively, with carbapenem resistance observed in 38.3% of *E. coli* and 44.2% of Klebsiella species isolates.

The identification of all *Staphylococcus aureus* isolates as MRSA is particularly concerning. This conforms to the report by Al Musawi *et al*. (2022), indicating a yearly 10% increase in MRSA prevalence in Saudi Arabia. Altered penicillin-binding proteins (PBPs) reduce their affinity for beta-lactam antibiotics, highlighting the widespread misuse of penicillins and cephalosporins (Eipa *et al*., 2023). Although these isolates remained sensitive to alternative antibiotics like vancomycin and linezolid, this sensitivity offers some hope. This is consistent with the study by Said *et al*. (2023), which reported >90% sensitivity. Similarly, all *Enterococcus faecium* isolates were found to be vancomycin-resistant, likely due to changes in cell wall structures driven by genes such as vanA or vanB (Lee *et al*., 2019). This high prevalence contrasts with other studies by Alhumaid *et al*. (2021) and Bazaid *et al*. (2021). Despite these alarming findings, alternative drugs for VRE, including daptomycin and linezolid (Chuang *et al*., 2014), demonstrated sensitivity in this study, offering promising options when vancomycin fails.

The discovery of isolates exhibiting both ESBL and CRE resistance is significant. Carbapenems, once considered last-resort antibiotics, are now compromised, with these strains evading multiple classes of antibiotics and severely limiting treatment options. This underscores the urgent need for

coordinated efforts, including infection control, antibiotic stewardship, and research into novel therapies, to combat this growing threat.

A particularly troubling finding is the high level of colistin resistance observed in all ESBL, CRE, and combined ESBL + CRE isolates. This rise in resistance can be attributed to the increased use of colistin as a last-resort antibiotic. Polymyxins, including colistin, were globally discontinued due to adverse reactions such as nephrotoxicity and neurotoxicity (Soman *et al*., 2020). However, they were reintroduced in Saudi hospitals to combat the continual increase in antimicrobial resistance (Alqasim, 2021; Wu *et al*., 2022). Studies in 2019 reported colistin as the most effective antibiotic against ESBL and CRE in Saudi Arabia (Khan *et al*., 2019). However, later studies by Aldawsari *et al*. (2020) and Taha *et al*. (2023) revealed alarmingly high resistance rates to colistin. Our study found 100% resistance to colistin in all isolates, including those with diverse resistant phenotypes, and 22.9% resistance in isolates with combined (ESBL + CRE) resistance phenotypes. In contrast, a 2022 study by Saleem *et al*. in Hail, a city near Najran, reported colistin susceptibility rates between 91.9% and 100%. This disparity highlights interesting regional differences, with Hail blending urban and rural areas, while Najran is primarily rural and near the Yemeni border. These distinctions underscore how AMR varies across regions (Skandalis *et al*., 2021). Rural regions struggle with access to quality healthcare, and factors such as climate and environment further contribute to the spread of resistant pathogens (Frost *et al*., 2019; Wu *et al*., 2024). In some regions, due to struggles with access to quality healthcare, patients access pharmacies more for over-the-counter drugs, where pharmacists prescribe antibiotics even when illegal (Bin Nafisah *et al*., 2017; Ajabnoor and Cooper, 2020).

Participants aged 50+ showed a higher prevalence of antibiotic-resistant phenotypes across various bacterial organisms. Aging leads to immunosenescence, making older adults more susceptible to infections. Chronic illnesses such as diabetes, cardiovascular diseases, and chronic obstructive pulmonary disease (COPD) further weaken host defenses, necessitating prolonged antibiotic use and increasing the risk of resistance. Additionally, older adults accumulate antibiotic exposure over their lifetime, creating selective pressure favoring resistant strains. Chronic conditions often require frequent medical interventions and the use of invasive medical devices, which provide entry points for resistant bacteria (Wezi, 2023).

Males exhibited higher prevalence rates for resistance phenotypes in organisms like *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecium*. This can be linked to increased exposure to hospital environments, invasive procedures, and occupational risks. Men often have higher rates of chronic conditions necessitating frequent hospitalizations and interventions that expose them to resistant bacteria. This conforms to results in Table 7 where male participants had higher prevalence in the ICU. Occupational factors, including manual labor and higher risk of injuries, increase their susceptibility to skin and soft tissue infections. Additionally, the Hajj pilgrimage organized in Saudi Arabia is attended mostly by men, potentially facilitating pathogen exchange from diverse regions (Alqasim, 2020; Alqasim, 2021). In contrast, females had a higher prevalence of *E. coli* resistance phenotypes (ESBL + Colistin), likely due to recurrent urinary tract infections (UTIs) and repeated antibiotic use. Women are biologically more prone to UTIs, leading to more frequent antibiotic prescriptions, which drive resistance (McLellan and Hunstad, 2016). Hormonal influences and behavioral factors also play a role, as females tend to seek healthcare more frequently, increasing their exposure to healthcare settings and potentially resistant pathogens (Edem *et al*., 2021).

High prevalence of resistance phenotypes was observed in the OPD and ICU, at 55.8% and 14.7%, respectively. The high prevalence in the OPD can likely be due to its role as a gateway for

community-acquired infections (Klepser *et al*., 2017). Patients with mild symptoms frequently visit the OPD, contributing to the spread of resistant bacteria. Overprescribing antibiotics in the OPD is common, as clinicians may prescribe antibiotics for viral infections or self-limiting conditions, further promoting resistance. Additionally, the high patient influx in the OPD increases exposure to resistant strains (King *et al*., 2018). However, for ICU, being a high-risk environment contributes significantly to resistance. At the ICU, critically ill patients undergoing invasive procedures, such as ventilation, catheterization, and surgery, are at increased risk of infections (Masoud, 2020). The use of broad-spectrum antibiotics is common in ICUs, as patients often receive potent antibiotics empirically, promoting the development and spread of resistant bacteria (Vincent *et al*., 2020).

Urine samples had the highest prevalence of resistance phenotypes, likely due to the common occurrence of UTIs and frequent collection of urine samples for diagnostic purposes. Catheterization and other invasive procedures increase the risk of resistant infections in urinary tracts (Khaparkuntikar *et al*., 2017). This also suggests that UTIs are a significant source of antibiotic resistance. All isolates and their respective resistance phenotypes had high prevalence in urine samples, except for *Acinetobacter baumannii* (ESBL + CRE + Colistin phenotype) and *Staphylococcus aureus* (MRSA phenotype) who had high prevalence in wound samples. This disparity indicates pathogens' ability to persist in wounds and their association with skin and soft tissue infections, often exacerbated by hospital-acquired infections.

Future studies should consider a multicenter approach and include community-acquired infections for a more comprehensive understanding. Further research should explore the molecular basis of resistance and investigate the role of antibiotic prescribing practices in the emergence of resistance. Additionally, studies assessing the clinical outcomes of patients infected with resistant strains and the effectiveness of alternative treatment regimens are needed to inform clinical management strategies.

References

Akereuke, U., Onwuezobe, I., Ekuma, A., Edem, E., Uko, N., Okon, R., ... & Ekpenyong, E. (2023). Molecular profile of metallo-β-lactamase producing bacterial isolates from clinical samples; South-South Nigeria perspective. *Mikrobiolohichnyi Zhurnal, 85*(6), 15–25.

Khaparkuntikar, M., Siddiqui, N., & Bhirud, P. (2017). Urinary tract infection in cancer patients at Government Cancer Hospital Aurangabad, India. *International Journal of Current Microbiology and Applied Sciences, 6*(5), 2259–2263. https://doi.org/10.20546/ijcmas.2017.605.251

Wezi, S. (2023). Why are we more prone to infection as we get older? *Medical Research Foundation.* https://www.medicalresearchfoundation.org.uk/news/stopping-the-spread-of-amr-by-reducing-the-impact-of-age-related-diseases

Jones, N., Mitchell, J., Cooke, P., Baral, S., Arjyal, A., Shrestha, A., & King, R. (2022). Gender and antimicrobial resistance: What can we learn from applying a gendered lens to data analysis using a participatory arts case study? *Frontiers in Global Women's Health, 3,* 745862. https://doi.org/10.3389/fgwh.2022.745862

Akinjogunla, O. J., Adefiranye, O. O., Edem, E. N., Adenugba, I. T., Ogboona, F. C., & Oshosanya, G. O. (2024). Antibiotic resistance profile, multidrug-, extensively drug-, and pandrug-resistant bacterial isolates: Hemagglutination and hemolytic activities against human erythrocytes. *Proceedings of the National Academy of Sciences, India, Section B: Biological Sciences.*

Bin Nafisah, S., Bin Nafesa, S., Alamery, A. H., Alhumaid, M. A., AlMuhaidib, H. M., & Al-Eidan, F. A. (2017). Over-the-counter antibiotics in Saudi Arabia, an urgent call for policymakers. *Journal of Infection and Public Health, 10*(5), 522–526. https://doi.org/10.1016/j.jiph.2016.09.016

Ajabnoor, A. M., & Cooper, R. J. (2020). Pharmacists' prescribing in Saudi Arabia: Cross-sectional study describing current practices and future perspectives. *Pharmacy (Basel, Switzerland), 8*(3), 160. https://doi.org/10.3390/pharmacy8030160

Masoud, Z. (2020). High infection prevalence among patients in the intensive care unit. *Infectious Disease Advisor.* https://www.infectiousdiseaseadvisor.com/news/infection-rates-in-icus-are-higher-than-previous-years/

Vincent, J., Sakr, Y., Singer, M., Martin-Loeches, I., Machado, F. R., Marshall, J. C., ... Angus, D. C. (2020). Prevalence and outcomes of infection among patients in intensive care units in 2017. *JAMA, 323*(15), 1478. https://doi.org/10.1001/jama.2020.2717

Skandalis, N., Maeusli, M., Papafotis, D., Miller, S., Lee, B., Theologidis, I., & Luna, B. (2021). Environmental spread of antibiotic resistance. *Antibiotics (Basel), 10*(6), 640. https://doi.org/10.3390/antibiotics10060640

Wu, J., Hu, Y., Perlin, M. H., Danko, D., Lu, J., Oliveira, M., ... Shi, T. (2024). Landscape of global urban environmental resistome and its association with local socioeconomic and medical status. *Science China Life Sciences, 67*(6), 1292–1301. https://doi.org/10.1007/s11427-023-2504-1

Frost, I., Van Boeckel, T. P., Pires, J., Craig, J., & Laxminarayan, R. (2019). Global geographic trends in antimicrobial resistance: The role of international travel. *Journal of Travel Medicine, 26*(8). https://doi.org/10.1093/jtm/taz036

Saleem, M., Syed Khaja, A. S., Hossain, A., Alenazi, F., Said, K. B., Moursi, S. A., ... Rakha, E. (2022). Molecular characterization and antibiogram of *Acinetobacter baumannii* clinical isolates recovered from the patients with ventilator-associated pneumonia. *Healthcare, 10,* 2210.

Aldawsari, A., Tawfik, K., & Al-Zaagi, I., Sr. (2020). Antimicrobial-resistant bacteria and prescription of antibiotics at a tertiary care hospital in Riyadh, Saudi Arabia. *Cureus, 12,* e12098.

Khan, M. A., Mohamed, A. M., Faiz, A., & Ahmad, J. (2019). Enterobacterial infection in Saudi Arabia: First record of *Klebsiella pneumoniae* with triple carbapenemase genes resistance. *Journal of Infection in Developing Countries, 13,* 334–341.

Said, K. B., AlGhasab, N. S., Alharbi, M. S., Alsolami, A., Bashir, A. I., Saleem, M., ... Alshamri, J. A. (2023). A sequalae of lineage divergence in *Staphylococcus aureus* from community-acquired patterns in youth to hospital-associated profiles in seniors implied age-specific host-selection from a common ancestor. *Diagnostics, 13,* 819.

Bazaid, A. S., Saeed, A., Alrashidi, A., Alrashidi, A., Alshaghdali, K., Hammam, S. A., ... Thallab, R. (2021). Antimicrobial surveillance for bacterial uropathogens in Ha'il, Saudi Arabia: A five-year multicenter retrospective study. *Infectious Drug Resistance, 14,* 1455–1465.

Alhumaid, S., Al Mutair, A., Al Alawi, Z., Alzahrani, A. J., Tobaiqy, M., Alresasi, A. M., ... Alismail, M. (2021). Antimicrobial susceptibility of gram-positive and gram-negative bacteria: A five-year retrospective analysis at a multi-hospital healthcare system in Saudi Arabia. *Annals of Clinical Microbiology and Antimicrobials, 20,* 43.

Al Musawi, S., Alkhaleefa, Q., Alnassri, S., Alamri, A. M., & Alnimr, A. (2022). Eleven-year surveillance of methicillin-resistant *Staphylococcus aureus* infections at an academic health center. *Journal of Preventive Medicine and Hygiene, 63,* E132–E138.

Kasanga, M., Shempela, D. M., Daka, V., Mwikisa, M. J., Sikalima, J., Chanda, D., & Mudenda, S. (2024). Antimicrobial resistance profiles of *Escherichia coli* isolated from clinical and environmental samples: Findings and implications. *JAC-Antimicrobial Resistance, 6*(2), dlae061. https://doi.org/10.1093/jacamr/dlae061

Regassa, B. T., Tosisa, W., Eshetu, D., Beyene, D., Abdeta, A., Negeri, A. A., ... Awoke, T. (2023). Antimicrobial resistance profiles of bacterial isolates from clinical specimens referred to Ethiopian Public Health Institute: Analysis of five-year data. *BMC Infectious Diseases, 23*(1), 798. https://doi.org/10.1186/s12879-023-08803-x

Torumkuney, D., Dolgum, S., Van Hasselt, J., Abdullah, W., & Keles, N. (2022). Country data on AMR in Saudi Arabia in the context of community-acquired respiratory tract infections: Links between antibiotic susceptibility, local and international antibiotic prescribing guidelines, access to medicine, and clinical outcome. *Journal of Antimicrobial Chemotherapy, 77*(Supplement_1), i70–i76. https://doi.org/10.1093/jac/dkac219

Mutair, A. A., Alhumaid, S., Alawi, Z. A., Zaidi, A. R. Z., Alzahrani, A. J., Al-Tawfiq, J. A., ... Al-Omari, A. (2021). Five-year resistance trends in pathogens causing healthcare-associated infections at a multi-hospital healthcare system in Saudi Arabia, 2015–2019. *Journal of Global Antimicrobial Resistance, 25,* 142–150. https://doi.org/10.1016/j.jgar.2021.03.009

McLellan, L. K., & Hunstad, D. A. (2016). Urinary tract infection: Pathogenesis and outlook. *Trends in Molecular Medicine, 22*(11), 946–957. https://doi.org/10.1016/j.molmed.2016.09.003

King, L. M., Fleming-Dutra, K. E., & Hicks, L. A. (2018). Advances in optimizing the prescription of antibiotics in outpatient settings. *BMJ,* k3047. https://doi.org/10.1136/bmj.k3047

Klepser, M. E., Dobson, E. L., Pogue, J. M., Labreche, M. J., Adams, A. J., Gauthier, T. P., ... Suda, K. J. (2017). A call to action for outpatient antibiotic stewardship. *Journal of the American Pharmacists Association, 57*(4), 457–463. https://doi.org/10.1016/j.japh.2017.03.013

Taha, R., Mowallad, A., Mufti, A., Althaqafi, A., Jiman-Fatani, A. A., El-Hossary, D., ... Kaaki, M. (2023). Prevalence of carbapenem-resistant Enterobacteriaceae in Western Saudi Arabia and increasing trends in the antimicrobial resistance of Enterobacteriaceae. *Cureus, 15,* e35050.

Kurdi, S., Faran, A., Eareeni, E., & others. (2020). Assessment of knowledge and attitude toward the new antibiotic dispensing law and its effect on antibiotic use in Saudi Arabia. *Saudi Pharmaceutical Journal, 28*(1), 58–67.

Higuita-Gutiérrez, L. F., Molina-Garcia, V., Acevedo Guiral, J., Gómez Cadena, L., Roncancio Villamil, G. E., & Jiménez Quiceno, J. N. (2020). Knowledge regarding antibiotic use among students of three medical schools in Medellin, Colombia: A cross-sectional study. *BMC Medical Education, 20*(1), 22.

Alqasim, A. (2021). Colistin-resistant Gram-negative bacteria in Saudi Arabia: A literature review. *Journal of King Saud University Science, 33*(8), 101610. https://doi.org/10.1016/j.jksus.2021.101610

Wu, X. L., Long, W. M., Lu, Q., Teng, X. Q., Qi, T. T., Qu, Q., ... Qu, J. (2022). Polymyxin B-associated nephrotoxicity and its predictors: A retrospective study in carbapenem-resistant

Gram-negative bacterial infections. *Frontiers in Pharmacology*, *13*, 672543. https://doi.org/10.3389/fphar.2022.672543

Soman, R., Bakthavatchalam, Y. D., Nadarajan, A., Dwarakanathan, H. T., Venkatasubramanian, R., & Veeraraghavan, B. (2021). Is it time to move away from polymyxins?: Evidence and alternatives. *European Journal of Clinical Microbiology & Infectious Diseases, 40*(3), 461–475. https://doi.org/10.1007/s10096-020-04053-w

Edem, E. N., Mbong, E. O., & Olaniyan, U. O. (2021). Environmental and human behavioral factors associated with vulvovaginal candidiasis among single and married women in Eket. *Global Journal of Infectious Diseases and Clinical Research, 7*(1), 37–42. https://doi.org/10.17352/2455-5363.000044

Al Yousef, S. A. (2016). Surveillance of antibiotic-resistant bacteria in King Khalid Hospital, Hafr Al-Batin, Saudi Arabia, during 2013. *Jundishapur Journal of Microbiology*, *9*(9), e19552.

Banawas, S. S., Alobaidi, A. S., Dawoud, T. M., AlDehaimi, A., Alsubaie, F. M., Abdel-Hadi, A., & Manikandan, P. (2023). Prevalence of multidrug-resistant bacteria in healthcare-associated bloodstream infections at hospitals in Riyadh, Saudi Arabia. *Pathogens, 12*(9), 1075.

Borgio, J. F., Rasdan, A. S., Sonbol, B., Alhamid, G., Almandil, N. B., & AbdulAzeez, S. (2021). Emerging status of multidrug-resistant bacteria and fungi in the Arabian Peninsula. *Biology, 10*(11), 1144.

Cheesebrough, M. (2010). *District laboratory in tropical countries* (2nd ed., Part 1). Cambridge University Press.

Magiorakos, A., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant, and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection, 18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Devi, L. S., Sardar, M., & Sharma, M. (2024). Concomitant carbapenem and colistin resistance among *Escherichia coli* and *Klebsiella pneumoniae* isolates from patients visiting a hospital in Haryana, India. *Medical Journal DY Patil Vidyapeeth, 17*, 160–167.

Edem, E. N., Onwuezobe, I. A., Ochang, E. A., Etok, C. A., & James, I. S. (2013). Antibiogram of bacterial isolates from the anterior nares and hands of healthcare workers in University of Uyo Teaching Hospital (UUTH) Uyo, Akwa Ibom State, Nigeria. *Journal of Bacteriology & Parasitology, 4*, 168.

Edem, E. N., Umoh, A., Olaniyan, U. O. O., & Anikpe, J. N. (2021). Genexpert MTB/RIF diagnostic yield of *Mycobacterium tuberculosis* and rifampicin resistance in Uyo, Nigeria. *Clinical Medicine, 3*(2), 1034.

Egwuatu, T., Ishola, O. D., & Oladele, O. E. (2021). The distribution of extended-spectrum beta-lactamase genes in fomites, healthcare workers, and patients from two hospitals in Lagos State, Nigeria. *Ife Journal of Science, 23*(2), 15–24.

Etok, C. A., Edem, E. N., & Ochang, E. (2012). Aetiology and antimicrobial studies of surgical wound infections in University of Uyo Teaching Hospital (UUTH) Uyo, Akwa Ibom State, Nigeria. *Scientific Reports*, 1, 341. https://doi.org/10.4172/scientificreports.341

Hsueh, P. R., Liu, C. Y., & Luh, K. T. (2002). Current status of antimicrobial resistance in Taiwan. *Emerging Infectious Diseases, 8*(2), 132–137.

Okon, R. S., Onwuezobe, I., Edem, E. N., Akereuke, U. E., Bonne, S., & Bawonda, E. O. (2023). Bacterial aetiologic profile and antibiotic response patterns of lower respiratory tract

infection (LRTI) among HIV/AIDS patients on highly active antiretroviral therapy (HAART) in Uyo, South-South Nigeria. *Mikrobiolohichnyi Zhurnal*, *68*(3), 185–195.

Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health*, *109*(7), 309–318.

Eipa, B. R., Islam, M. R., Sultana, R., Alam, S. T., Mehjabin, T., Bushra, N. N. H., ... Uddin, M. A. (2023, June 18). Determination of the antibiotic susceptibility pattern of gram-positive bacteria causing UTI in Dhaka, Bangladesh. *arXiv*. https://arxiv.org/abs/2306.10553

Miteu, G. D., Achinebiri, P., Raghunathan, N., & Sankaran, S. (2023). Closing potential drivers of antimicrobial resistance: Last-resort antimicrobials with the potential of being misused, the way forward – A short communication. *Annals of Medicine and Surgery*, *85*(6), 3226–3231. https://doi.org/10.1097/ms9.0000000000000760

Sarkar, M. M. H., Afroz, J., Jubyda, F. T., Sharmin, S., Faruq, M. J., Dey, A. K., ... Farzana, T. (2019). Community-acquired multidrug-resistant clinical strains from tracheal aspirates of patients in hospital settings in Dhaka, Bangladesh. *Research Square*. https://doi.org/10.21203/rs.2.18948/v1

Rousham, E., Unicomb, L., Wood, P., Smith, M., Asaduzzaman, M., & Islam, M. A. (2018). Spatial and temporal variation in the community prevalence of antibiotic resistance in Bangladesh: An integrated surveillance study protocol. *BMJ Open*, *8*(4), e023158. https://doi.org/10.1136/bmjopen-2018-023158

Kohinke, R. M., & Pakyz, A. L. (2017). Treatment of vancomycin-resistant enterococci: Focus on daptomycin. *Current Infectious Disease Reports*, *19*(10), 33. https://doi.org/10.1007/s11908-017-0589-2

Chuang, Y. C., Wang, J. T., Lin, H. Y., & Chang, S. C. (2014). Daptomycin versus linezolid for treatment of vancomycin-resistant enterococcal bacteremia: Systematic review and meta-analysis. *BMC Infectious Diseases*, *14*, 687. https://doi.org/10.1186/s12879-014-0687-9

Lee, T., Pang, S., Abraham, S., & Coombs, G. W. (2019). Antimicrobial-resistant CC17 *Enterococcus faecium*: The past, the present, and the future. *Journal of Global Antimicrobial Resistance*, *16*, 36–47. https://doi.org/10.1016/j.jgar.2018.08.016