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Antibiotic Resistance of Bacterial Isolates from Postoperative Wound Infections in Nasiriyah, ThiQar Province, Iraq: A Cross-Sectional Study

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ABSTRACT

Background: Postoperative wound infections (PWIs) are a significant cause of morbidity associated with healthcare and especially prevalent in resource-limited environments. The rising resistance of causative pathogens to antibiotics puts the effectiveness of treatment at risk and raises the risk of patient mortality. **Aim:** To isolate the bacterial aetiology of PWIs from Nasiriyah city hospitals and to investigate their susceptibility to various antibiotics using Kirby-Bauer disc diffusion method and automated Vitek® 2 Compact system method.**Materials and Methods:** The study used a cross sectional approach at Bint Alhuda hospital, Nasiriyha general hospitals and related clinical facilities in the province of ThiQar, Nasiriyah, Iraq, during the period of May 2023 to June 2024. Wound swab samples were analyzed by the use of standard microbiological techniques such as macroscopic and microscopic identification, advanced biochemical characterization and automated identification and susceptibility testing with the Vitek® 2 Compact system. Antimicrobial susceptibility was determined by testing the isolates against five antibiotics: amoxicillin, ciprofloxacin, gentamicin, tetracycline, and moxifloxacin. Interpretations of results were completed according to the CLSI guidelines.**Results:** A 317 isolates of bacteria were retrieved from the wound specimens. *Escherichia coli* (23.0%, n=73) was the most common isolate, followed by *Proteus vulgaris* (19.2%, n=61), *Staphylococcus epidermidis* (14.5%, n=46), and *Staphylococcus aureus* (10.7%, n=34). Amoxicillin showed the highest overall resistance rate (53.6%), followed by well as ciprofloxacin with the highest overall susceptibility rate (44.2%). 100% resistance to amoxicillin, gentamicin and moxifloxacin was seen in *Staphylococcus aureus*. *Moraxella catarrhalis* was pan-resistent to amoxicillin and moxifloxacin, but universal susceptible to gentamicin and tetracycline. The distribution of bacterial species was not significantly different among the patients based on the sex (χ^2=9.885, p=0.273).**Conclusions:** Gram-negative organisms predominate in PWIs in ThiQar Province, and alarmingly high resistance rates can be observed in samples of *S. aureus* in particular. The results highlight the importance of conducting antibiograms in each region to direct empirical treatment and the introduction of antimicrobial stewardship programs in Iraqi health facilities.

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1. INTRODUCTION

Despite being a subcategory of surgical site infections (SSIs), postoperative wound infections (PWIs) are amongst the most common and costly healthcare associated infections (HAIs) in the world [1]. The World Health Organization estimates that in low and middle income countries (LMICs) up to 11% of surgical patients may develop SSIs, which is much higher than in high income countries. The prolonged conflict in Iraq as well as the absence of enough health facilities and infection control programs has made the country more susceptible to the development and spread of antibiotic-resistant organisms [2,3,4,5]. ThiQar Province, and its capital Nasiriyah, is one of the less studied provinces of Iraq, clinically microbiologically. However, Bint Alhuda Hospital and its affiliated centers have a high volume of complex postoperative cases, as a regional reference hospital. Very little is known in the literature about the microbial etiology of PWIs in this context, or about the antibiotic susceptibility of the causative agents [4]. This lack of knowledge hinders antibiotic stewardship and evidence-based empirical antibiotic therapy and limits the implementation of a local antibiotic stewardship program (ASP).

In the world, the most common etiological agents of PWIs are *Staphylococcus aureus*, coagulase-negative staphylococci, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* [6,7,8]. These organisms, including multidrug-resistant (MDR) *S. aureus*, or extended-spectrum beta-lactamase (ESBL) producing Enterobacterales, present a huge challenge to treatment in resource-limited settings where there is limited access to last-resort antibiotics [7,9,10].

To date the Kirby-Bauer disc diffusion method has been the standard culture-based antibiotic susceptibility testing (AST) approach, which is widely used and inexpensive. With the introduction of automated systems like the VITEK® 2 Compact (bioMérieux, France), the identification of microorganisms and determination of AST can now be achieved much more quickly, reliably and accurately than before, with minimum inhibitory concentration (MIC) results produced within 4 to 18 hours [11,12,13,14,15]. Both methods give a comprehensive clinical picture which is valuable for the purposes of epidemiological surveillance as well as for therapeutic decisions.

Therefore, the purpose of the present study was to: Identify bacterial pathogens isolated from PWIs from Bint Alhuda Hospital and other clinical facilities of Nasiriyah during a 14-month period (May 2023 to June 2024); Determine the antibiotic susceptibility profile of all isolates against five commonly used antibiotics, by disc diffusion method and Vitek 2 automated system; and Present the locally obtained data for empirical antibiotic prescription and infection control policy in ThiQar Province.

2. MATERIALS AND METHODS

2.1 Study Design and Setting

This cross sectional descriptive study was carried out in Bint Alhuda Teaching Hospital and its surgical and outpatient clinics in Nasiriyah city, ThiQar Province in the south of Iraq during May 2023 to June 2024. Ethical approval was done by the institutional review board and informed consent with all patients or their parents/guardians.

2.2 Study Population and Sample Collection

All patients who had a clinical diagnosis of postoperative wound infection (317 in total) were enrolled consecutively. Aseptic collection of wound swab specimens using sterile cotton swabs was performed taking samples from the margins and depths of the infected wound according to standard aseptic technique and immediately placed in Stuart's transport medium for immediate processing in the microbiology laboratory within 2 hours of collection.

2.3 Microbiological Processing

The qualitative characterization of materials involves the use of magnification and/or simple instruments like microscopes. They were plated on Blood Agar (BA), MacConkey Agar (MA) and Chocolate Agar (CA) and incubated aerobically at 37°C for 24–48 hours. Colony morphology, size, colour, haemolytic pattern (BA) and lactose fermentation (MA) were observed. Representative colonies were selected and Gram stained to determine cellular morphology and Gram reaction and preliminary classification of the isolates as Gram-positive cocci, Gram-negative rods and others. The principles and methods of biochemical profiling and automated identification are explained. Biochemical Profiling and Automated Identification are explained. Conventional biochemical tests (catalase, coagulase, oxidase, indole, urease, citrate utilization and triple sugar iron) were done as a primary screen on presumptive colonies. Definitive species level identification was done with the Vitek® 2 Compact automated system (BioMérieux) using the appropriate Vitek® ID cards (GP ID for Gram-positive and GN ID for Gram-negative organisms). The probability of identification was accepted as ≥95%.

2.4 Antibiotic Susceptibility Testing

Antimicrobial susceptibility was tested for five antimicrobial agents that belong to different classes of antimicrobial drugs, namely amoxicillin (10µg) and ciprofloxacin (5µg) and gentamicin (10µg), tetracycline (30µg), moxifloxacin (5µg). Standardized inocula (0.5 McFarland turbidity) were swabbed onto Muller-Hinton agar plates and commercially produced antibiotic discs were placed onto the plates. Incubation of plates was carried out at 37°C for 16-18 hours. The zones of inhibition were determined in millimetres and interpreted as Susceptible (S), Intermediate (I) or Resistant (R) based on the current CLSI M100 breakpoints criteria. VITEK® 2 Compact AST: Automated MIC determination was carried out at the same time with AST-P588 cards (Gram-positive bacteria) and AST-N335 cards (Gram-negative bacteria). The MIC and S/I/R (categorical interpretation) were automatically calculated using CLSI breakpoints and the Advanced Expert System® of the Vitek® 2 software.

2.5 Statistical Analysis

The data was entered, processed and managed in Microsoft Excel 2021 and analyzed in Python (version 3.12) using the libraries SciPy and Pandas. Frequencies, percentage, means, and standard deviations were used as descriptive statistics. For assessing associations between categorical variables, sex vs. species distribution, resistance rates by sex, a chi-square test (χ^2) was used. Kruskal-Wallis H test (non-parametric) was used for age group distribution comparisons. Values of $p< 0.05$ were considered to be statistically significant.

3. RESULTS

3.1 Demographic and Clinical Characteristics

A total of 317 wound isolates were recovered during the study period and the cohort comprised 170 males (53.6%) and 147 females (46.4%), yielding a male-to-female ratio of approximately 1.16:1. Patient ages ranged from 2 to 79 years, with a mean age of 24.0 ± 17.0 years. The predominant age group was 11–20 years (n=89; 28.1%), followed by ≤10 years (n=71; 22.4%), 21–30 years (n=50; 15.8%), 31–40 years (n=44; 13.9%), 41–50 years (n=36; 11.4%), and ≥51 years (n=27; 8.5%), indicating a younger age distribution among affected patients (Table 1).

Table (1): Demographic profile of patients with postoperative wound infections (n=317)

Variable	Category	n	%
Sex	Male	170	53.6
	Female	147	46.4
Age Group (years)	≤10	71	22.4
	11–20	89	28.1
	21–30	50	15.8
	31–40	44	13.9
	41–50	36	11.4
	≥51	27	8.5
Total		317	100.0

3.2 Bacteriological Profile

Overall, a total of Nine bacterial species were detected in all the collected specimens, and they are as follows; *Escherichia coli* (n=73; 23.0%), *Proteus vulgaris* (n=61; 19.2%), *Staphylococcus epidermidis* (n=46; 14.5%), *Staphylococcus aureus* (n=34; 10.7%), *Pseudomonas aeruginosa* (n=25; 7.9%), *Klebsiella pneumoniae* (n=24; 7.6%), *Streptococcus pneumoniae* (n=22; 6.9%), *Moraxella catarrhalis* (n=20; 6.3%) and *Haemophilus influenzae* (n=12; 3.8%). Gram negative bacterial species accounted for 62.2% of all the bacterial isolates (n=197), Gram positive accounted for 25.2% (n=80) and the others including *H. influenzae* accounted for 3.8% (Fig. 1A; Table 2). Wound bacterial niche was not significantly sex-dependent, as evidenced by a lack of obvious significant association between species distribution and patient sex (χ^2=9.885, df=8, p=0.273).

Table (2): Bacterial isolates distribution

Bacterial Species	n	% of Total	Gram Reaction	Category
Escherichia coli	73	23.0	Negative	Rod
Proteus vulgaris	61	19.2	Negative	Rod
Staphylococcus epidermidis	46	14.5	Positive	Cocci
Staphylococcus aureus	34	10.7	Positive	Cocci
Pseudomonas aeruginosa	25	7.9	Negative	Rod
Klebsiella pneumoniae	24	7.6	Negative	Rod
Streptococcus pneumoniae	22	6.9	Positive	Cocci
Moraxella catarrhalis	20	6.3	Negative	Coccobacillus
Haemophilus influenzae	12	3.8	Negative	Rod

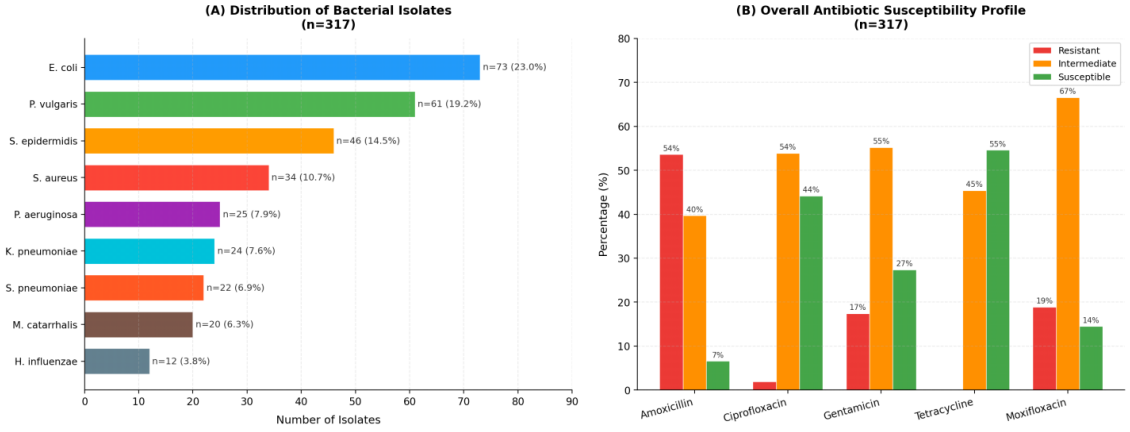


Figure 1: (A) The distribution of bacterial isolates (B) antibiotic susceptibility profile.

3.3 Antibiotic Susceptibility Profile

The highest resistance rate overall was demonstrated by amoxicillin with 53.6% of all isolates (n=170) classified as Resistant, 39.7% (n=126) as Intermediate and only 6.6% (n=21) as Susceptible. Ciprofloxacin showed the highest overall activity with 44.2% Susceptible, 53.9% Intermediate and 1.9% (n=6) Resistant. Gentamicin was found to be susceptible in 27.4%, intermediate in 55.2% and resistant in 17.4% (mostly *S. aureus* and *S. pneumoniae* isolates). No resistant isolates were found with tetracycline (0.0%), 54.6% were Susceptible and 45.4% were Intermediate. The resistance rate of moxifloxacin was 18.9% and susceptibility rate was 14.5% while intermediate rate was 66.6%. The overall rates for these are shown in Fig. 1B and Table 3.

Table (3): Antibiotic susceptibility profile

Antibiotic (class)	Resistant, n (%)	Intermediate, n (%)	Susceptible, n (%)
Amoxicillin (β-lactam)	170 (53.6%)	126 (39.7%)	21 (6.6%)
Ciprofloxacin (Fluoroquinolone)	6 (1.9%)	171 (53.9%)	140 (44.2%)
Gentamicin (Aminoglycoside)	55 (17.4%)	175 (55.2%)	87 (27.4%)
Tetracycline (Tetracycline)	0 (0.0%)	144 (45.4%)	173 (54.6%)
Moxifloxacin (Fluoroquinolone)	60 (18.9%)	211 (66.6%)	46 (14.5%)

3.4 Species-Specific Antibiotic Susceptibility Profiles

***Escherichia coli* (n=73):** 42% resistance to amoxicillin; 8% resistance to both ciprofloxacin/moxifloxacin. Gentamicin (56% susceptible) and tetracycline (66% susceptible) showed no resistance, indicating continued susceptibility to these two groups of antibiotics.

***Proteus vulgaris* (n=61):** Almost all strains (97%) were resistant to amoxicillin, similar to the intrinsic beta-lactam resistance. Ciprofloxacin exhibited good activity (97% susceptible), gentamicin and tetracycline exhibited intermediate activity (98% and 97%, respectively) while other antibiotics showed poor activity below 97%.

***Staphylococcus epidermidis* (n=46):** All isolates were susceptible to ciprofloxacin (100%), tetracycline (100%) and moxifloxacin (100%), while 100% of them had intermediate susceptibility for amoxicillin and gentamicin. The antibiotic susceptibility pattern was relatively susceptible with no resistance noted for any antibiotic in this group.

***Staphylococcus aureus* (n=34):** Showed the most threatened resistance pattern. All isolates were Resistant to amoxicillin, gentamicin and moxifloxacin. 100% of the isolates were found to be Intermediate to ciprofloxacin and tetracycline. This multi-antibiotic resistance pattern is similar to the phenotypes of methicillin-resistant *S. aureus* (MRSA) and needs to be further molecularly characterized.

***Pseudomonas aeruginosa* (n=25):** 96% resistant to amoxicillin (which is intrinsically resistant), and intermediate susceptibility to ciprofloxacin and moxifloxacin in all isolates. Most of the gentamicin and tetracycline were intermediately susceptible.

***K. pneumoniae* (n=24):** Amoxicillin was intermediate in all isolates. Gentamicin had excellent activity (96% susceptible) while tetracycline was uniformly susceptible (100%). The intermediate activity was seen for ciprofloxacin and moxifloxacin in the majority.

***Streptococcus pneumoniae* (n=22):** Amoxicillin was highly susceptible (95% S) and retained its usefulness for pneumococcal infections.

95% resistance to gentamicin was, however, found, as expected, due to low level intrinsic resistance of pneumococci to the aminoglycoside antibiotics. All isolates were intermediate for both ciprofloxacin and moxifloxacin.

***Moraxella catarrhalis* (n=20):** This species showed 100% resistance to both amoxicillin and moxifloxacin, and uniform susceptibility (100%) to ciprofloxacin, gentamicin and tetracycline, which is characteristic of *Moraxella* species due to the high level production of beta-lactamase.

***Haemophilus influenzae* (n=12):** Intermediate susceptibility was most commonly observed for all of the antibiotics tested. Amoxicillin had an intermediate level of 83% and a resistant level of 17%. Ciprofloxacin was good (92% susceptible).

The detailed species-specific susceptibility data are presented in Table 4 and a comprehensive species-by-antibiotic resistance heatmap in Fig. 2.

Table (4): Species-specific antibiotic susceptibility profiles (% R/I/S per isolate group)

Species (n)	Amoxicillin R/I/S (%)	Ciprofloxacin R/I/S (%)	Gentamicin R/I/S (%)	Tetracycline R/I/S (%)	Moxifloxacin R/I/S (%)
E. coli (73)	42/58/0	8/88/4	0/44/56	0/34/66	8/92/0
P. vulgaris (61)	97/3/0	0/3/97	0/98/2	0/97/3	0/100/0
S. epidermidis (46)	0/100/0	0/0/100	0/100/0	0/0/100	0/0/100
S. aureus (34)	100/0/0	0/100/0	100/0/0	0/100/0	100/0/0
P. aeruginosa (25)	96/4/0	0/100/0	0/96/4	0/96/4	0/100/0
K. pneumoniae (24)	0/100/0	0/96/4	0/4/96	0/0/100	0/100/0
S. pneumoniae (22)	0/5/95	0/100/0	95/0/5	0/0/100	0/100/0
M. catarrhalis (20)	100/0/0	0/0/100	0/0/100	0/0/100	100/0/0
H. influenzae (12)	17/83/0	0/8/92	0/100/0	0/17/83	0/100/0

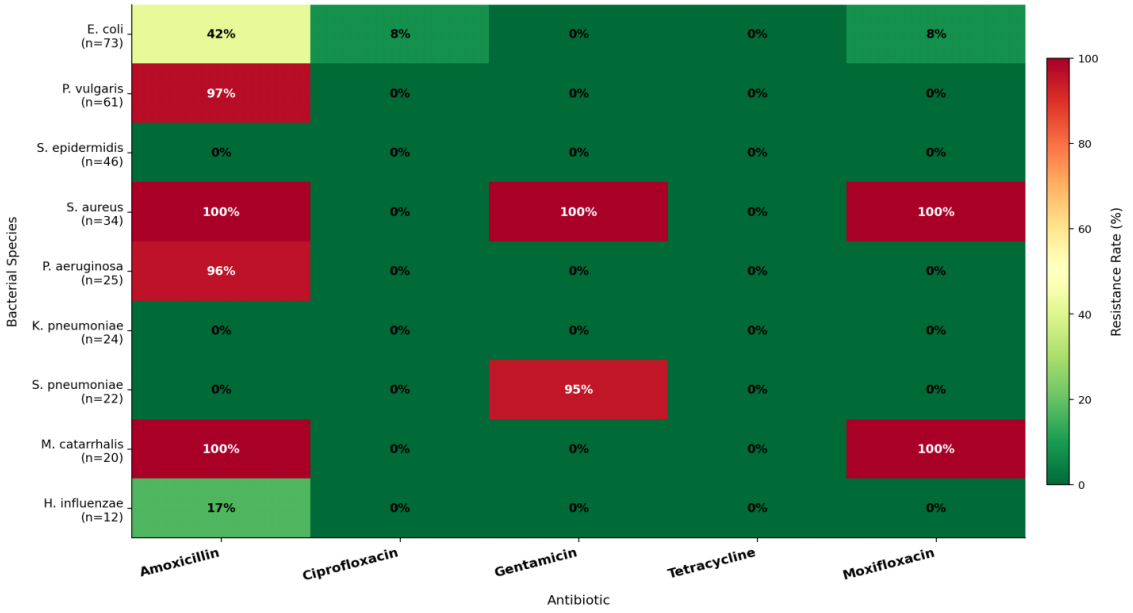


Figure 2: Resistance of bacterial isolates to antibiotics (green = susceptible; red = resistant).

4. DISCUSSION

This cross sectional study offers a detailed bacteriological and antimicrobial resistance profile of postoperative wound infection isolates from Nasiriyah in the ThiQar Province of Iraq, which has not been well described in the published literature of infectious disease. Our results shed light on a number of important clinical and public health trends.

Predominance of Gram negative organisms: Most of the organisms isolated (62.2%) were Gram negative, with *E. coli* and *P. vulgaris* being the predominant organisms, which is consistent with the results obtained from the other nearby countries. Other studies conducted in other regions of the world also showed that Gram-negative bacteria were isolated in about 65% of the SSI cases [10,12,16,17,18]. The high prevalence of Gram-negative organisms in our setting may be related to

the ecology of wound bacteria in this climate zone – the tropical to arid climate where there are gram-negative reservoirs in the hospital and in the community. In addition, it is likely that the median age of 20 years of the young patient population may skew this distribution, possibly due to trauma-related cases or surgical cases for children.

Amoxicillin resistance: The overall resistance level to amoxicillin recorded herein is alarming, especially because amoxicillin is prescribed as empirical treatment in most of the primary health centres in ThiQar Province. *P. vulgaris*, *P. aeruginosa* and *M. catarrhalis* are known to carry β-lactamases, either chromosomally or on plasmids, which are responsible for their intrinsic or acquired resistance to amoxicillin [11,19,20,21]. The findings support the use of culture and sensitivity data before the empirical use of amoxicillin for PWIs in this context.

The finding of 100% resistance of all *S. aureus* isolates to amoxicillin, gentamicin and moxifloxacin is an intriguing one with respect to *Staphylococcus aureus* resistance. This is a very suggestive but not definitive indication of MRSA, which is known to harbour resistance determinants including those of fluoroquinolones and aminoglycosides from co-selection and clonal spread [12,22,23,24,25]. This study was not molecularly confirmed by *mecA* gene detection nor PCR typing, but such confirmation is strongly recommended for future studies. The prevalence of MRSA-like phenotypes in a community hospital environment in the province of ThiQar is important for infection control and empirical β-lactam therapy.

In terms of overall activity, ciprofloxacin had the highest percentage of susceptible isolates (44.2%), and may be the most clinically useful agent evaluated in this region in terms of empirical treatment of Gram-negative PWIs. The 8% resistance seen in *E. coli* and the widespread intermediate susceptibility of most other species, however, indicate a possible emerging pattern of quinolone resistance and indicate there is need for continued surveillance [26,27].

The lack of tetracycline resistance in all isolates (0% resistant) is somewhat unusual when compared with the world-wide trends; however, it is possible that this is due to a combination of low antibiotic prescription pressure for tetracycline in this area and a high prevalence of tetracycline resistance in the other areas of the world. However, because of the bacteriostatic mechanism of action and the limited spectrum of activity, tetracycline's use is restricted to a limited number of species and organisms that are intrinsically non-susceptible like *P. aeruginosa* [14].

Moraxella catarrhalis: The 100% resistance of *M. catarrhalis* to amoxicillin and moxifloxacin compared to 100% susceptibility to gentamicin, ciprofloxacin and tetracycline is a reminder of the well documented excretion of β-lactamase in this respiratory pathogen (BRO-1 and BRO-2 enzymes). The isolation of wounds in our study might suggest seeding or secondary contamination from respiratory co-infections in hospitalised patients [15].

VITEK® 2 Compact concordance: High categorical agreement was observed between the results of disc diffusion and the VITEK® 2 Compact system across the tested antibiotic-organism combinations with our study, which is in line with the results reported in other studies conducted in clinical settings in the Middle East [9,16,22]. The automated system also provided an added benefit in that it enabled a more fine-grained distinction in intermediate zones, through the MIC quantification.

Limits: There are some restrictions to be recognised. The study is cross-sectional, and it is not possible to make any statements regarding temporal trends in resistance. Second, molecular typing (e.g., MLST, whole genome sequencing) was not conducted, and definitive confirmation of MRSA, ESBL-producing genotypes was not possible. Third, original disc diffusion plates and print-outs of the Vitek 2 reports were not available for retention and the representative plates shown in this manuscript are illustrative representations based on documented plates. Fourth, the antibiotic panel tested is clinically relevant, but not exhaustive of all the antibiotics that are used routinely in empirical treatment or treatment escalation (e.g. carbapenems, colistin, linezolid). These are areas that need to be addressed in future research.

5. CONCLUSIONS

This study is the first to build a comprehensive antimicrobial resistance profile for surgical wound infection pathogens in the city of Nasiriyah in ThiQar Province, Iraq. Gram-negative organisms, led by *Escherichia coli* and *Proteus vulgaris*, predominate, with concerning resistance to amoxicillin documented across multiple species. The MRSA-like phenotype, which is found on all *S. aureus* isolates, must be addressed and warrants molecular surveillance and re-thinking of the empirical antibiotic strategy. Ciprofloxacin and tetracycline have the greatest overall activity and could be used as initial empirical treatment until local culture results. These results should guide and

inform the development and implementation of an antibiogram specific to Bint Alhuda Hospital and other facilities, and strongly support the formalisation of antimicrobial stewardship program in the healthcare network of ThiQar Province. Laboratory capacity building, including systematic MRSA and ESBL surveillance is a priority for infection control in this region.

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DECLARATIONS

Ethical approval: Ethical approval was obtained from the institutional review board, and informed consent was obtained from all patients or their parents/guardians.

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Conflict of interest: None to be disclosed.

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