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Prevalence and Antimicrobial Resistance of Escherichia coli Isolated from Patients with Nosocomial Infections

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Abstract— Background*: Gram-negative infections are important public-health problem due to their relation with high morbidity, and antimicrobial resistance. In this study, the distribution of bacterial isolates was determined and in particular the presence of Escherichia coli, as well as the analysis of antimicrobial resistance profile.* ***Methods:*** *A prospective cohort study was conducted on115 patients with hospital-acquired infections attended to privet hospitals in Al-Samawa (Iraq), from January to July 2026. Bacterial isolates were categorized according to species, patient age, gender, hospital ward, and infection type. Antimicrobial susceptibility testing was performed for E. coli isolates against several antibiotics.* ***Results****: E. coli was the most prevalent isolate, accounting for 38 cases (33.0%), followed by K. pneumoniae (27.8%), P. aeruginosa (20.0%), A. baumannii complex (12.2%), and Proteus spp. (7.0%). Patients aged 60 years or older constituted the largest age group (36.5%), followed by those aged 40–59 years (31.3%; p =0.0004). Females represented 59.1% of cases and males 40.9% (p =0.050). The ICU had the highest overall number of isolates (30.4%). Urinary infections were most common (38.3%), and 63.2% of E. coli isolates were associated with urinary infections. Among E. coli, susceptibility was highest to meropenem (89.5%), amikacin (84.2%), and nitrofurantoin (78.9%). The greatest resistance was observed to erythromycin (97.4%), cephalothin (86.8%), ceftriaxone and cefotaxime (73.7% each), and ceftazidime (68.4%).* ***Conclusion:*** *E. coli was the leading bacterial isolate associated with hospital-acquired infections in the studied patients. The high resistance to several commonly used antimicrobials emphasizes the need for ongoing surveillance, evidence-based empirical therapy, antimicrobial stewardship, and effective infection-prevention strategies.*

Keywords— *Bacterial isolates; Gram-negative;E. coli; antimicrobial susceptibility.*

I. INTRODUCTION

Hospital-acquired infections (HAIs) remain a major challenge for healthcare systems [1] because they are associated with prolonged hospitalization, increased treatment costs, antimicrobial use, and adverse clinical outcomes [2,3]. Gram-negative bacteria are especially relevant in causing HAIs because they can be resistant to several antimicrobial agents and can survive for long periods in the healthcare setting [4]. Of these organisms, Escherichia coli (E. coli) are commonly involved in urinary, wound, respiratory, and blood stream infections [5,6].

The E. coli is responsible for high prevalence in HAIs due to a number of reasons including its capacity to colonize the gastrointestinal system, and the tendency to develop antibiotic resistance [7,8]. This bacterium has become multidrug resistant and is a source of increased difficulty and failure to treat in Iraq where antibiotics have been overused and misused [9]. Furthermore, poor infection control practices within health care systems may also play a role in the spread of these pathogens, leading to "hard-to-control" hospital acquired infections [10].

Hospital-associated E. coli isolates are becoming resistant to antimicrobials [11], making empirical treatment more challenging and the importance of local surveillance data is even more important [12]. Resistance patterns may vary according to geographic location, hospital ward, infection type, and patient characteristics [13, 14].

The present study aimed to determine the prevalence and distribution of bacterial isolates associated with hospital-acquired infections among patients attending hospitals. Particular emphasis was placed on the distribution of E. coli according to hospital ward, infection type, and antimicrobial susceptibility pattern.

II. METHODS

A. Patients and study design

In this prospective cohort study participated 115 patients attended private hospitals in Al-Samawa, Al- Muthanna (Iraq) between January and July 2026 to identify bacterial isolates associated with hospital-acquired infections.

It included all ages and both genders that consented to participate in an informed manner; for younger ages, parental consent was obtained, while those deemed unfit to participate were excluded.

The study population comprised patients with hospital-associated infections; bacterial isolates recovered from the patients were classified according to bacterial species, hospital ward, and type of infection.

B. Specimen collection and transport

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Clinical specimens were collected from patients with suspected hospital-acquired infections according to the suspected site of infection. These included urine specimens for urinary infections; wound swabs or burn-wound specimens for wound and burn infections; respiratory specimens such as sputum, endotracheal aspirates, or bronchoalveolar lavage specimens for respiratory infections; and blood cultures for bloodstream infections. Specimens should be collected aseptically before the initiation or modification of antimicrobial therapy whenever possible and transported promptly to the microbiology laboratory using appropriate sterile containers or transport media.

Specimens were inoculated onto appropriate bacteriological culture media and incubated under suitable atmospheric and temperature conditions. Depending on the type of specimen, the culture media may have included:

Blood agar, for recovery of a broad range of bacterial pathogens and assessment of colony morphology and hemolysis; MacConkey agar, for isolation of Gram-negative bacilli and differentiation of lactose-fermenting organisms; Cystine–lactose–electrolyte-deficient agar, particularly for urine cultures; Eosin methylene blue agar, where available, for differentiation of lactose-fermenting coliforms and presumptive recognition of E. coli; Chocolate agar, when required for respiratory specimens or organisms requiring enriched media; and Selective or differential media for wound and burn specimens, according to the laboratory's routine protocol.

Growth was determined by the appearance of the colonies, colony counts (where applicable), pigment production, hemolysis, smell, and presence of mixed growth after incubation. In case of urine specimens, the presence of significant bacteriuria depends on the quantitative colony counts as well as the clinical picture. Blood cultures to be monitored for growth, and sub cultured if positive.

C. Bacterial Identification

The gram staining was conducted as preliminary classification test and to find the purity of the isolate prior to biochemical identification and susceptibility testing. Gram staining was used to examine the different colonies. Gram-negative bacilli or coccobacilli are expected to appear in the culture as E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii complex, and Proteus spp. Identification of the bacteria was carried out using a combination of colony morphology, Gram staining and standard biochemical tests or a commercially available automation system.

- *Identification of Escherichia coli*

Presumptive E. coli isolates were characterized as Gram-negative, lactose-fermenting bacilli producing pink colonies on MacConkey agar. Identification may be confirmed using the following tests: Oxidase: negative; Indole: positive; Methyl red: positive; Voges–Proskauer: negative; Citrate utilization: negative; Urease: generally negative; Motility: positive; and Triple sugar iron agar: acid production in the butt and slant, usually with gas production and without hydrogen sulfide. A commercial identification system, such as API20E, VITEK, or MALDI-TOF mass spectrometry, may also be used when available.

- *Identification of Klebsiella pneumoniae*

K. pneumoniae is typically identified as a nonmotile, Gram-negative, lactose-fermenting bacillus producing large, mucoid colonies on MacConkey agar. Common biochemical characteristics include: Oxidase: negative; Indole: negative for typical K. pneumoniae; Citrate: positive; Urease: positive; Voges–Proskauer: positive; and Motility: negative.

- *Identification of Pseudomonas aeruginosa*

P. aeruginosa may be recognized by its non-lactose-fermenting colonies on MacConkey agar and characteristic pigment production on some media. Confirmatory tests may include: Gram-negative slender bacilli; Oxidase: positive; Catalase: positive; Motility: positive; Growth at approximately 42°C; Production of a grape-like odor; and Production of pyocyanin or pyoverdine, when present.

- *Identification of Acinetobacter baumannii complex*

Members of the A. baumannii complex are typically identified as Gram-negative coccobacilli that may appear as smooth, non-lactose-fermenting or weakly lactose-fermenting colonies. Relevant tests include: Oxidase: negative; Catalase: positive; Non-motility; Non-fermentative reaction on carbohydrate media; and Growth on routine blood and MacConkey media.

Because phenotypic methods may not reliably distinguish A. baumannii from closely related members of the A. baumannii complex, definitive species-level identification requires an automated identification platform, MALDI-TOF, or molecular testing where available.

- *Identification of Proteus species*

Proteus spp. may be identified by characteristic swarming growth on blood agar and non-lactose-fermenting colonies on MacConkey agar. Supporting biochemical tests include: Gram-negative bacilli; Oxidase: negative; Urease: positive; Phenylalanine deaminase: positive; Motility: positive; Hydrogen sulfide production: variable depending on species; and Swarming motility on blood agar.

D. Classification according to hospital ward

After identification, each isolate was linked to the ward from which the patient was sampled. The isolates were then categorized as originating from the ICU, surgery, medicine, or burns ward. The ward-specific results were calculated by counting the number of isolates of each bacterial species recovered from each ward and expressing these values as frequencies and percentages.

E. Classification according to infection type

The site of infection was determined from the clinical diagnosis and the type of specimen submitted to the laboratory. Isolates were grouped into urinary, wound and burn, respiratory, or bloodstream infections. The corresponding laboratory tests included: Urinary infections: urine culture, colony counting, Gram staining, and biochemical identification of significant isolates. Wound and burn infections: direct Gram staining, aerobic culture, colony morphology, and biochemical identification. Respiratory

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infections: assessment of specimen quality, direct Gram staining, culture on blood and enriched media, and bacterial identification; and Bloodstream infections: blood-culture incubation, detection of positive cultures, Gram staining, subculture, and biochemical or automated identification.

F. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on 38 confirmed E. coli isolates. Reporting the susceptible, intermediate, and resistant classification is done using the standard Kirby–Bauer disk diffusion method on Mueller–Hinton agar. A fresh culture of bacteria was used to prepare a pure bacterial suspension for this procedure.

It was evenly inoculated on the surface of Mueller–Hinton agar and then antimicrobial disks were applied. After incubation under standard conditions, the inhibition zone around the disk was determined in mm. The susceptibility results were summarized as the number and percentage of E. coli isolates in each category.

G. Quality control

Quality-control testing should be performed with reference strains to confirm the accuracy of culture media, antimicrobial disks, and susceptibility procedures. Appropriate control strains may include: Escherichia coli ATCC25922 for routine antimicrobial susceptibility testing; and Additional reference strains where required for media and biochemical-test validation. Only results obtained from pure cultures and quality-controlled procedures should be included in the final analysis.

H. Data analysis

The results were processed using descriptive statistics with SPSS software and tabulated with frequencies (n) and percentages (%). The chi-square test was applied to assess patient variance according to age group and gender. A p-value less than 0.05 were set statistically significant.

III. RESULTS

A total of 115 bacterial isolates associated with hospital-acquired infections were confirmed in this study. E. coli was the most frequently isolated organism; accounting for 38 isolates (33.0%). This was followed by K. pneumoniae with 32 isolates (27.8%), P. aeruginosa with 23 (20.0%), A. baumannii complex with 14 (12.2%), and Proteus spp. with 8 isolates (7.0%) as illustrated in Table (1).

TABLE I. Proportion of bacterial isolates with nosocomial infections.

Bacterial Species	**Isolates**	
	(n)	(%)
Escherichia coli	38	33.0%
Klebsiella pneumoniae	32	27.8%
Pseudomonas aeruginosa	23	20.0%
***Acinetobacter baumannii* complex**	14	12.2%
***Proteus* spp.**	8	7.0%
Total	115	100%

The largest proportion of patients was aged 60 years or older, comprising 42 patients (36.5%). Patients aged 40–59 years accounted for 36 cases (31.3%), while 25 patients (21.7%) were aged 20–39 years. The smallest age group was patients' younger than 20 years, with12 cases (10.4%). The distribution across age groups was statistically significant according to reported analysis (p =0.0004) as shown in Table (2). Otherwise, females represented 68 patients (59.1%), whereas males accounted for 47 patients (40.9%). The reported p-value for the gender distribution was 0.050 (Table 3).

TABLE II. Bacterial isolates according to age group of participants

Age Group (years)	**Patients**		**p-value**
	(n)	(%)	
< 20	12	10.4%	0.0004
20 – 39	25	21.7%	
40 – 59	36	31.3%	
≥ 60	42	36.5%	
Total	**115**	**100%**	

TABLE III. Bacterial isolates according to gender of participants.

Gender	**Patients**		**p-value**
	(n)	(n)	
Females	68	59.1%	0.050
Males	47	40.9%	
Total	115	100%	

The highest frequency of bacterial isolates was obtained from the ICU, which accounted for 35 isolates (30.4%), followed by the surgery and medicine wards; each with 31 isolates (27.0%). The burns ward contributed18 isolates (15.6%).

Among the 38 E. coli isolates, the largest number was recovered from the medicine ward, with15 isolates (39.5%), followed by the surgery ward with14 isolates (36.8%). Six E. coli isolates (15.8%) were obtained from the ICU and three (7.9%) from the burns ward.

The distribution of other organisms varied by ward, A. baumannii complex was predominantly isolated in the ICU, where 9 of 14 isolates (64.3%) were recovered. P. aeruginosa was most common in the burns ward, accounting for 10 of 23 isolates (43.5%), whereas Proteus spp. were most frequently isolated in the surgery ward, with 4 of 8 isolates (50.0%) as displayed in Table (4).

In Table (5), urinary infections were the most frequent infection category, accounting for 44 cases (38.3%), followed by wound and burn infections with 32 cases (27.8%), respiratory infections with 26 cases (22.6%), and bloodstream infections with13 cases (11.3%).

E. coli was predominantly associated with urinary infections, which accounted for 24 of 38 E. coli isolates (63.2%). Eight isolates (21.1%) were recovered from wound and burn infections, four (10.5%) from bloodstream infections, and two (5.3%) from respiratory infections.

K. pneumoniae was also most frequently associated with urinary infections, although it showed a broader distribution across infection types. In contrast, P. aeruginosa was primarily associated with wound and burn infections (52.2%), while A. baumannii complex was most frequently associated with respiratory infections (50.0%).

Among 38 E. coli isolates, meropenem showed the highest

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activity, with 34 isolates (89.5%) classified as susceptible. Amikacin was also highly active, with 32 susceptible isolates (84.2%), followed by nitrofurantoin, to which 30 isolates (78.9%) were susceptible.

Intermediate susceptibility was generally low, ranging from1 to 4 isolates depending on the antimicrobial agent.

TABLE IV. Bacterial isolates according to hospital wards

Bacterial Species	ICU	Surgery	Medicine	Burns	Total
E. coli	6 (15.8%)	14 (36.8%)	15 (39.5%)	3 (7.9%)	38 (33.0%)
K. pneumoniae	12 (37.5%)	8 (25.0%)	10 (31.3%)	2 (6.2%)	32 (27.8%)
P.aeruginosa	7 (30.4%)	4 (17.4%)	2 (8.7%)	10 (43.5%)	23 (20.0%)
***A. baumannii* complex**	9 (64.3%)	1 (7.1%)	1 (7.1%)	3 (21.4%)	14 (12.2%)
***Proteus* spp.**	1 (12.5%)	4 (50.0%)	3 (37.5%)	0 (0.0%)	8 (7.0%)
Total	35 (30.4%)	31 (27.0%)	31 (27.0%)	18 (15.6%)	115 (100.0%)

TABLE V. Bacterial isolates according to infections.

Bacterial Species	Urinary	Wound & Burn	Respiratory	Blood stream	Total
E. coli	24 (63.2%)	8 (21.1%)	2 (5.3%)	4 (10.5%)	38 (33.0%)
K.pneumoniae	12 (37.5%)	6 (18.8%)	10 (31.3%)	4 (12.5%)	32 (27.8%)
P. aeruginosa	2 (8.7%)	12 (52.2%)	7 (30.4%)	2 (8.7%)	23 (20.0%)
***A. baumannii* complex**	1 (7.1%)	4 (28.6%)	7 (50.0%)	2 (14.3%)	14 (12.2%)
***Proteus* spp.**	5 (62.5%)	2 (25.0%)	0 (0.0%)	1 (12.5%)	8 (7.0%)
Total	44 (38.3%)	32 (27.8%)	26 (22.6%)	13 (11.3%)	115 (100%)

The highest resistance rates were observed for erythromycin, with 37 isolates (97.4%) resistant, cephalothin with 33 isolates (86.8%), ceftriaxone and cefotaxime with28 isolates each (73.7%), and ceftazidime with 26 isolates (68.4%). Resistance was also substantial to cefepime (60.5%), doxycycline (57.9%), chloramphenicol and gentamicin (47.4% each). Finally, Cefoxitin showed a comparatively lower resistance rate of 31.6% as shown in Table (6).

TABLE VI. Antimicrobial susceptibility of E. coli isolates (n=38).

Antibiotic	Susceptible	Intermediate	Resistant
Meropenem	34 (89.5%)	1 (2.6%)	3 (7.9%)
Chloramphenicol	16 (42.1%)	4 (10.5%)	18 (47.4%)
Doxycycline	13 (34.2%)	3 (7.9%)	22 (57.9%)
Ceftazidime	10 (26.3%)	2 (5.3%)	26 (68.4%)
Cefoxitin	23 (60.5%)	3 (7.9%)	12 (31.6%)
Cefepime	12 (31.6%)	3 (7.9%)	23 (60.5%)
Nitrofurantoin	30 (78.9%)	2 (5.3%)	6 (15.8%)
Ceftriaxone	8 (21.1%)	2 (5.3%)	28 (73.7%)
Erythromycin	0 (0.0%)	1 (2.6%)	37 (97.4%)
Amikacin	32 (84.2%)	2 (5.3%)	4 (10.5%)
Cefotaxime	9 (23.7%)	1 (2.6%)	28 (73.7%)
Cephalothin	3 (7.9%)	2 (5.3%)	33 (86.8%)
Gentamicin	17 (44.7%)	3 (7.9%)	18 (47.4%)

IV. DISCUSSION

This study demonstrates that E. coli was the predominant bacterial species among 115 isolates recovered from studied patients. It accounted for one-third of all isolates, exceeding the proportions observed for K. pneumoniae, P. aeruginosa, A. baumannii complex, and Proteus spp. The predominance of E. coli is clinically important because this organism is a common cause of urinary and other healthcare-associated infections [15] and may serve as an important indicator of antimicrobial pressure within hospital settings [16]. This finding is consistent with previous studies [17-19].

The results also confirmed that the older age group was the most affected, with 36.5% of cases recorded in the older age group (60 years and over), and another 31.3% in the age group from 40 to 59 years. This may be due to the higher susceptibility of older patients due to comorbidities, decreased immunity, invasive procedures, longer hospital stays, and more exposure to antimicrobial therapy [20,21]. The statistically significant age distribution is reflective of the observation that hospital-associated infections were not evenly distributed among the age groups.

The study population consisted of more females than males, and the difference was near the margin of significance as indicated by the reported p-value of 0.050. This finding that a greater number of female patients may be related at least in part with the substantial contribution of urinary infection, particularly as E. coli was strongly associated with urinary infection in this study [22]. However, the data available are not sufficient to define the gender dimorphism or to draw a firm conclusion on its cause.

Most bacterial isolates were obtained from the ICU, and this is similar to the clinical features of intensive care units, where invasive procedures are more likely to be performed, mechanical ventilation is used, the length of hospital stay increased, and patients receive broad-spectrum antibiotics [23,24]. The high prevalence of A. baumannii complex in the ICU is notable, because this organism is often associated with critical care environments, and can survive on hospital surfaces and equipment [25]. E. coli on the other hand was most commonly found in the medicine and surgery ward, reflecting that the distribution of E. coli was dependent on the type of patient and treatment that was undertaken in these

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wards [26].

Another aspect of the infection-type distribution that should be noted is the clinical significance of E. coli. The urinary tract had the highest percentage of infections in total, and accounted for almost 25% of all E. coli isolates. This is biologically and clinically reasonable as E. coli is a significant urinary pathogen and hospital urinary infections are often related to urinary catheterization and other urological procedures [27]. It is important to note that different bacterial species can have different clinical and ward associated patterns, as seen with the concentration of P. aeruginosa in wound and burn infections [28] and of A. baumannii complex in respiratory infections [29].The antimicrobial resistance results showed a worrisome resistance pattern in the E. coli isolates. Meropenem, amikacin and nitrofurantoin had the highest in vitro susceptibility (89.5%, 84.2% and 78.9%, respectively).

The high activity of nitrofurantoin is of special interest in urinary infections, but is used clinically only in suitable lower urinary tract infections and needs to be guided by site and severity of infection [30]. Substantial activities were found in Meropenem and Amikacin, but their use should be carefully monitored as resistance to these agents could be further promoted.

Resistance to multiple cephalosporins was prevalent, and is interesting to note that resistance rates of 60.5%, 68.4% and 73.7% were measured for the resistance to ceftazidime, cefotaxime and ceftriaxone respectively. The results indicate significant level of resistance to extended spectrum cephalosporins among the E. coli isolates studied. A high resistance to cephalothin and erythromycin makes the use of these antibiotics against the isolates examined even more restricted [31]. Susceptibility data alone cannot confirm the presence of an extended-spectrum beta-lactamase or other genes responsible for resistance, so the results do not provide specific resistance mechanisms, and this is a conclusion that would need further laboratory testing [32].

The results confirm the importance of the constant monitoring, proper specimen collection, use of antimicrobial agents, and apply infection control measures in hospital. Treatment protocols should be monitored on an empirical basis, based on local susceptibility data as well as general treatment guidelines.

V. Conclusion

E. coli was the most common bacterial isolate found in hospital-acquired infections. The majority of patients were older, and females prevailed among the patients. There were more isolates from the ICU than from other wards; E coli were most commonly isolated from the medicine and surgery wards. Urinary infections were the most common type of infection and represented most of the E. coli infections. Furthermore, it was observed that there was a significant proportion of E. coli isolates in the hospital environment that were resistant to antimicrobial drugs and the importance of regular susceptibility testing, local resistance surveillance, antimicrobial stewardship and improved infection control practices.

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