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RESEARCH ARTICLE

KNOWLEDGE AND PRACTICE IN USING THE ANTIMICROBIAL SUSCEPTIBILITY TEST TOWARDS *ENTEROBACTERIACEAE* ISOLATED FROM URINARY TRACT INFECTION

Mohammed Abdullah Al-Khawi[1], Saleh S. Bahaj[1], Huda Zaid Al-Shami[1], Hassan Abdulwahab Al-Shamahy[1,2], Eshrak Adulmalik Al-Gunaid[3]

[1]*Medical Microbiology and Clinical Immunology Department, Faculty of Medicine and Health Sciences, Sana'a University.*
[2]*Departement of Basic Sciences, Faculty of Dentistry, Sana'a University, Republic of Yemen.*
[3]*The National Center of Public Health Laboratories (NCPHL), Sana'a, Yemen.*

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***Address for Correspondence:**
Dr. Hassan A. Al-Shamahy, Faculty of Medicine and Heath Sciences, Sana'a University, Yemen. Tel: +967-770299847;
E-mail: *hassanalshamahii@gmail.com*

Abstract

Background and aims: Antimicrobial resistance (AMR) is recognized as an urgent worldwide problem, particularly in developing countries like Yemen. Clinical microbiology laboratories play an essential role in guiding appropriate antimicrobial therapy through antimicrobial susceptibility testing (AST). This study aims to assess the knowledge and practices of antimicrobial susceptibility testing in public and private laboratories in Sana'a, Yemen, focusing on bacterial isolates from urinary tract infections (UTIs).
Materials and Methods: A cross-sectional study was conducted, analyzing 220 AST reports on positive urine cultures from public and private laboratories in Sana'a. The appropriateness of antimicrobial agent selection for testing and reporting susceptibility results was evaluated against CLSI M100-Ed32, 2022 guideline. Additionally, a standardized questionnaire was used to assess laboratory personnel's knowledge and practices related to AST.
Results: The study identified significant discrepancies in antimicrobial susceptibility testing and reporting practice between private and public laboratories in Sana'a, Yemen. Findings indicate a significant gap in the adherence to CLSI guidelines, with low testing and reporting rates for primary appropriate antimicrobial agents and over-reporting of inappropriate agents.
Conclusions: The study identified significant gaps in knowledge and adherence to international AST standards. Selective reporting is not being implemented. Therefore, a national antimicrobial program, including AST's unified guidelines, regular training in laboratory workers, and the creation of accurate internal and external measures to ensure accuracy and reliability of AST results.
Keywords: Antimicrobial susceptibility testing (AST), appropriateness of antimicrobial agents, CLSI guidelines, knowledge and practice, Urinary tract infection (UTI).

INTRODUCTION

Antimicrobial resistance (AMR) is recognized as an urgent worldwide problem, AMR has become increasing constantly and the choices of antibiotics for multi-drug resistant organisms become scarce day by day worldwide and in Yemen[1-7]. In 2019, an estimated 4.95 million deaths were associated with AMR, and 1.27 million deaths were attributable to bacterial drug-resistant infections globally[7,8]. According to data from the Institute for Health Metrics and Evaluation, among a total of 204 countries, Yemen ranked 74th in terms of the mortality rate associated with AMR in 2019. In that year, Yemen experienced 3,900 deaths that were directly linked to AMR, and 16,200 deaths were associated with AMR[9].

AMR arise generally due to genetic changes either by mutation or gene transfer. Moreover, some bacteria can share their resistance genes lead to emerging resistance phenotypes among wide variety of bacteria[10]. Overuse and misuse of antimicrobial agents in different life sectors such as agriculture and food industries as well as in medical sector accelerate AMR more common in third world countries as Yemen[3,4,7,11,12]. The role of inappropriate use of antimicrobial agents and AMR has been well recognized in health care facilities, communities and countries[5,6]. AMR changes in time, so frequent update of knowledge about the local

bacterial etiology and susceptibility patterns is recommended to trace any change in time[7,13-15,16].

In order to guarantee the appropriate use of antibiotics and to reduce AMR, World Health Organization (WHO) classified the antibiotic into three groups Access, Watch and Reserve (AWaRe) classification. Access group, characterized by their narrow spectrum activity, shows the lowest resistance potential. Watch group, antibiotics of this group have higher resistance potential than access and includes critically important antimicrobials. They are advised primarily for patients with severe clinical conditions or for infections likely to involve pathogens resistant to Access antibiotics. Reserve group, includes the last option for treatment of confirmed or suspected infections due to multi drug-resistant organisms, and should be used only when all other options have failed[17,18]. Clinical microbiology laboratories play an essential role in profiling the antibiotic susceptibility patterns of bacterial pathogens and conducting surveillance of AMR. These laboratories generate vital data that helps track the emergence of AMR and guiding physicians to select the most appropriate antibiotics for treatment[19-23].

Urinary tract infections (UTIs) are one of the most prevalent types of infections in Yemen (UTI study Yemen). UTIs often lead individuals to seek medical attention[16]. UTIs rank as the second most prevalent type of infection among all infectious diseases[24]. According to the Disease Burden and Long-Term Trends of UTIs report, the estimated cases of UTIs in the year 2019 were 404.61 million globally. Additionally, the report indicates that the number of deaths attributed to UTIs worldwide in 2019 was estimated to be 236,790[25]. UTIs are primarily caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*[16, 26,27]. In Sana'a city, *E. coli* was the most common isolate from community-acquired UTIs, while *K. pneumoniae* was the predominant isolate from hospital-acquired UTIs. Additionally, *S. aureus* was the most frequently isolated pathogen in catheter-associated UTIs. More than 90% of the pathogens isolated from nosocomial UTI specimens exhibited resistance to broad-spectrum Penicillins, Cephalosporin, Quinolones and Macrolides[28,29].

Antimicrobial susceptibility test (AST) is only performed for positive urine culture that demonstrate significant bacterial growth[30]. Accurate AST is essential for guiding physicians to select the most appropriate antibiotics for treatment. When susceptibility testing is performed correctly and standard guidelines are followed, it helps ensure that first-line or second-line antibiotic options are utilized whenever possible[31]. In contrast, inaccurate AST and failure to adhere to approved standard procedures can lead to inaccurate reporting. This, in turn, can mislead physicians and potentially contribute to the development of AMR[32].

Antimicrobial susceptibility results are reported in accordance with national laboratory standardized guidelines[33]. Susceptibility result is not reported for every drug, but only for appropriate drug-microorganism combinations. Appropriate AST reporting should also consider the specific site of the infection and the patient's clinical status such as, the patient's age, history of allergies, and pregnancy status[30,33]. In countries with limited resources, clinical laboratories face major challenges due to the lack of national standardized practice guidelines and standard operating procedures. The absence of these critical tools makes it difficult for these laboratories to consistently implement best practices and ensure quality assurance in their operations[34].

There was no previous study in Yemen for evaluation of the knowledge and practice in use AST. Moreover, Yemen lacks both a national antimicrobial guideline and a specific guideline for AST and reporting. Therefore, the main aim of the study is to assess the knowledge and practice in using of antimicrobial susceptibility test towards *Enterobacteriaceae* bacterial isolates from urinary tract infections in Sana'a city, Yemen. In addition, compare the local practice of selecting antimicrobial agents for susceptibility testing in private and public laboratories with the guidelines provided by Clinical and Laboratory Standards Institute (CLSI M100 "Performance Standards for Antimicrobial Susceptibility Testing"); and create a new form for reporting antimicrobial susceptibility test results.

MATERIALS AND METHODS

Study design: This study was a cross sectional KAP study.

Study population and study area: Patient reports for AST on positive urine cultures were obtained from the main public and private medical microbiology laboratories in Sana'a City, Yemen.

Inclusion criteria: This study focused on positive urine culture and susceptibility testing reports for *E. coli*, *Klebsiella* species, *Proteus* species, as these were the most common uropathogens.

Exclusion criteria

- Reports of uropathogens other than those specified in the inclusion criteria.
- Duplicate or repeated reports for the same patient.
- Reports of uropathogens isolated by only one laboratory.
- Reports of urine culture without AST result

Sample size: The sample size that met the inclusion criteria and was subsequently included in this study consisted of 220 reports. Sample size was calculated by using Epi Info application, based on the population size more than 100,000, confidence interval 95%, confidence limits 5%, and expected frequency 85%.

Data collection: To evaluate local practices in selecting antimicrobial agents for susceptibility testing, AST reports on positive urine cultures were collected from several private and public laboratories in Sana'a City. Additional essential information regarding knowledge and practices in using AST for bacterial isolates from UTIs was collected using a standardized questionnaire. This questionnaire included questions on guidelines, methods, criteria for interpreting results,

quality control standards used in testing and reporting, and patient data accompanying the sample collection.

Methods: A total of 220 AST reports on positive urine cultures, covering the period from January 4, 2023, to June 13, 2023, were included in this study. Of these, 109 reports were obtained from private laboratories, while 111 were collected from public laboratories. Each urine culture episode was examined to assess the appropriateness of the selected antimicrobial agents for testing and reporting the susceptibility results of the isolated bacteria. This evaluation was based on CLSI M100-Ed32, 2022 reference guideline, which was the most current version during the sampling period. The appropriateness of the antimicrobial agent selection was categorized based on the organism/antimicrobial agent combination as follows: Appropriate agents for routine, primary testing and reporting susceptibility results, appropriate agents for routine testing but only selective reporting, inappropriate agents for routine testing, inappropriate unclassified agents (those with no interpretive breakpoints available for the isolated bacteria and antimicrobial agent combination), inappropriate agents for testing susceptibility due to intrinsic resistant factors, and inappropriate agents for reporting on organisms isolated from UTIs. The appropriateness of selecting antimicrobial agents for testing and reporting susceptibility of each bacterial isolate was compared in detail between private and public laboratories. This comparison was conducted at both the individual antimicrobial agent level and across each category in general.

Patient data, specifically age, as well as the collected AST reports, were used to investigate the reporting rates of quinolones for patients under 18 years old. Additionally, essential information related to urine culture and susceptibility testing procedures, as well as results interpretation criteria, was gathered from all participating laboratories using a standardized questionnaire. This supplementary data was analyzed to assess overall knowledge and practices, extending beyond just the selection of antimicrobial agents.

Statistical analysis: The collected data were encoded and entered into a computer for statistical analysis, which was conducted using SPSS (Statistical Package for the Social Sciences), version 24, released in 2016. Cross-tabulation was employed to observe and compare the appropriateness of antimicrobial agent selection between private and public laboratories. For statistical comparison, Odds Ratio, 95% Confidence Interval (C.I), Chi-Square (χ^2), and *p*-value were utilized. An Odds Ratio greater than 1, a χ^2 value of 3.84 or greater, and a *p*-value of less than 0.05 were considered statistically significant.

Ethics consideration: Consent was obtained from all laboratories participating in this study. Each laboratory was informed that participation was voluntary and that they could withdraw at any time without providing a reason. This study was approved by the Ethical Committee of the Faculty of Medicine and Health Sciences.

RESULTS

Distribution of bacterial isolates: Gram-negative bacteria were the most commonly isolates, accounting 170 (77.3%), while Gram-positive bacteria comprised 50 (22.7%). Among Gram-negative bacteria, *Enterobacteriaceae* were the most prevalent, 147 (66.8%). Within the *Enterobacteriaceae*, *E. coli* was the most common uropathogen, representing 115 (52.27%), followed by *Klebsiella* species with 29 (13.18%) and *Proteus* species with 3 (1.36%). *P. aeruginosa* was identified in 23 (10.5%). Among Gram-positive bacteria, *Staphylococci* were predominant, 40 (18.2%), including 35 (15.9%) *S. aureus* and 5 (2.3%) *S. saprophyticus*. *Enterococci* were identified in 10 (4.5%).

Selection of antimicrobial agents: According to the responses from laboratories participating in the study, all of them indicated that CLSI is the reference guidelines they rely on for AST. So, this study considered CLSI M100-2022 criteria for analyzing the susceptibility data that collected from these laboratories and categorized the appropriateness of selection of antimicrobial agents into the following categories: (1) Appropriate agents for routine, primary testing and reporting susceptibility. These agents are considered the first and primary options to be selected for routine testing and reporting susceptibility result, (2) Appropriate agents for testing but only selective reporting susceptibility results. Agents of this category are considered the second options that can be tested routinely alongside the primary agents but their reporting may be selective in cases when the isolated bacteria demonstrate resistance to the primary agents or when the primary agents are not suitable for treatment, (3) Inappropriate agents for routine testing. This category includes alternative or supplemental agents that may be evaluated selectively for unusual cases when all appropriate agents are not the optimal therapeutic options, (4) Inappropriate unclassified agents that are not belong to the above categories and have no interpretive criteria for inhibition zone diameter or MIC breakpoints. Some of these agents susceptibility results can be predicted from the results of closely related agents of the same class. For example, the susceptibility of *staphylococci* to a wide array of β-lactam agents can be deduced by testing only penicillin and either Cefoxitin or Oxacillin. Routine testing of other β-lactam agents, except Ceftaroline, is not recommended, (5) Inappropriate agents for testing susceptibility due to intrinsic resistant factors, and (6) Inappropriate agents that are not routinely reported on organisms isolated from urinary tract.

Selection of antimicrobial agents for testing susceptibility of *Enterobacteriaceae*: A wide range of 63 different antimicrobial agents were utilized across 147 reports, with varied frequencies of usage. A total of 2,594 antimicrobial agents were employed, (1,587 agents across 72 reports from private laboratories, with an average of 22 agents per report and 1,007 agents across 75 reports from public laboratories, averaging 13 agents per report). Antimicrobial agents listed in Table 2A, are considered the first and primary options

to be selected for routine testing and reporting of *Enterobacteriaceae*. Nitrofurantoin had the highest utilization rates in both private and public laboratories, with rates of 72/72 (100%) and 69/72 (95.8%) respectively. Gentamicin followed with utilization rates of 48/72 (66.7%) in private laboratories and 54/75 (72%) in public laboratories. In contrast, Cefazolin had the lowest selection rates among both private and public laboratories, with rates of 15/72 (20.8%) and 2/75 (2.7%) respectively. The selection of Fosfomycin, Tobramycin, and Trimethoprim was limited to private laboratories, with relatively low rates of 22/63 (34.9%), 10/72 (13.9%), and 12/72 (16.7%) respectively.

Table 1A: Distribution of bacterial isolates by age group and gender, between private and public laboratories.

Age Group (Years)	Lab. Ownership	*E. coli*		*Klebsiella* species		*Proteus* species		*P. aeruginosa*		*S. aureus*		*S. saprophyticus*		*Enterococci*		Total	
		M	F	M	F	M	F	M	F	M	F	M	F	M	F		
<18	Private		9							1						10	24
	Public		5	2	2			3			2					14	
18-50	Private	10	28	1	4			3	3	2	12	1		3	5	72	138
	Public	15	17	7	4	3		6	2	4	6		2			66	
>50	Private	9	7	3	1			2		3				1	1	27	58
	Public	6	9	2	3			3	1		5	1	1			31	
Total	Private	19	44	4	5	0	0	5	3	6	12	1	0	4	6	109	220
	Public	21	31	11	9	3	0	12	3	4	13	1	3	0	0	111	
Total, No.(%)		115 (52.27%)		29 (13.18%)		3 (1.36%)				35 (15.9%)		5 (2.3%)					
		147 (66.8%)						23 (10.5%)				40 (18.2%)		10 (4.5%)			
		170 (77.3%)										50 (22.7%)				220 (100%)	

M = Male; F = Female

Private laboratories exhibited a statistically significant preference for selecting Cefazolin and Trimethoprim-Sulfamethoxazole. Specifically, for Cefazolin, the odds ratio was 9.6, the *p*-value was 0.001, and the χ^2 value was 11.9. For Trimethoprim-Sulfamethoxazole, the odds ratio was 4.2, the *p*-value was 0.001, and the χ^2 value was 16.9. In total, private laboratories showed a statistically significant preference for selecting the primary appropriate agents. The odds ratio of 1.8 indicates that private laboratories had a 1.8 times higher likelihood of selecting the primary appropriate agents compared to public laboratories. This difference was found to be statistically significant, as supported by the *p*-value of 0.001 and the χ^2 value of 23.4.

The antimicrobial agents listed in Table 2B can be tested routinely alongside the primary agents mentioned in Table 2A. However, their reporting may be selective in cases when the isolated bacteria demonstrate resistance to the primary agents or when the primary agents are not suitable for treatment. Amoxicillin-Clavulanate was the most widely used antimicrobial agent in both private and public laboratories, with utilization rates of 64/72 (88.9%) and 68/75 (90.7%) respectively. Ceftriaxone followed closely with utilization rates of 59/72 (81.9%) in private laboratories and 64/75 (85.3%) in public laboratories. The utilization rates of Ciprofloxacin were nearly equal in both private and public laboratories, with rates of 48/72 (66.7%) and 51/75 (68%) respectively. However, there was significant variation in the utilization rates of Levofloxacin. It was utilized at a rate of 63/72 (87.5%) in private laboratories and 40/75 (53.3%) in public laboratories, with a significant odds ratio of 6.1, a *p*-value of 0.001, and a χ^2 value of 20.4. Overall, private laboratories demonstrated a statistically significant preference for selecting the majority of agents listed in Table 2B, with a significant odds ratio of 3, a *p*-value of 0.001 and a χ^2 value of 165.5. According to the results presented in Table 2–C, private laboratories exhibited a statistically significant performance for selecting inappropriate agents compared to public laboratories. These findings were supported by the significant odds ratio of 1.8, a *p*-value of 0.001, and a χ^2 value of 68.1. The antimicrobial agents listed in Table 2B can be tested routinely alongside the primary agents mentioned in Table 2–A. However, their reporting may be selective in cases when the isolated bacteria demonstrate resistance to the primary agents or when the primary agents are not suitable for treatment. Amoxicillin-Clavulanate was the most widely used antimicrobial agent in both private and public laboratories, with utilization rates of 64/72 (88.9%) and 68/75 (90.7%) respectively. Ceftriaxone followed closely with utilization rates of 59/72 (81.9%) in private laboratories and 64/75 (85.3%) in public laboratories. The utilization rates of Ciprofloxacin were nearly equal in both private and public laboratories, with rates of 48/72 (66.7%) and 51/75 (68%) respectively. However, there was significant variation in the utilization rates of Levofloxacin. It was utilized at a rate of 63/72 (87.5%) in private laboratories and 40/75 (53.3%) in public laboratories, with a significant odds ratio of 6.1, a *p*-value of 0.001, and a χ^2 value of 20.4.

Table 2A: Selection of appropriate antimicrobial agents for routine, primary testing and reporting susceptibility of *Enterobacteriaceae* according to CLSI M100-2022.

Antimicrobial Agent	Lab. Ownership	Selected		Unselected		Total	Odds Ratio	95% C.I.		χ^2	*P-value*
		N	%	N	%			Lower	Upper		
Ampicillin[1]	Private	21	33.3	42	66.7	63	1.0	0.5	2.2	0.01	0.944
	Public	18	32.7	37	67.3	55					
	Total	39	33.1	79	66.9	118					
Cefazolin	Private	15	20.8	57	79.2	72	9.6	2.1	43.7	11.9	0.001
	Public	2	2.7	73	97.3	75					
	Total	17	11.6	130	88.4	147					
Trimethoprim-Sulfamethoxazole	Private	53	73.6	19	26.4	72	4.2	2.1	8.4	16.9	0.001
	Public	30	40.0	45	60.0	75					
	Total	83	56.5	64	43.5	147					
Nitrofurantoin[2]	Private	72	100.0	0	0.0	72	0.8	0.4	1.6	3.1	0.08
	Public	69	95.8	3	4.2	72					
	Total	141	97.9	3	2.1	144					
Gentamicin	Private	48	66.7	24	33.3	72	0.7	0.5	0.8	0.5	0.483
	Public	54	72.0	21	28.0	75					
	Total	102	69.4	45	30.6	147					
Tobramycin	Private	10	13.9	62	86.1	72				11.2	0.001
	Public	0	0.0	75	100.0	75					
	Total	10	6.8	137	93.2	147					
Trimethoprim	Private	12	16.7	60	83.3	72				13.6	0.001
	Public	0	0.0	75	100.0	75					
	Total	12	8.2	135	91.8	147					
Fosfomycin[3]	Private	22	34.9	41	65.1	63				22.5	0.001
	Public	0	0.0	52	100.0	52					
	Total	22	19.1	93	80.9	115					
Total	Private	253	45.3	305	54.7	558	1.8	1.4	2.3	23.4	0.001
	Public	173	31.2	381	68.8	554					
	Total	426	38.3	686	61.7	1112					

Results of Ampicillin testing can be used to predict results for Amoxicillin. *Klebsiella* species are intrinsically resistant to Ampicillin so, 29 cases of *Klebsiella* species were excluded. *Proteus* speciesare intrinsically resistant to Nitrofurantoin so, 3 cases of *Proteus* species were excluded. Fosfomycin is appropriate for testing and reporting of *E. coli* urinary tract isolates only and should not be used with other species of *Enterobacteriaceae*.

Overall, private laboratories demonstrated a statistically significant preference for selecting the majority of agents listed in Table 2B, with a significant odds ratio of 3, a *p*-value of 0.001 and a χ^2 value of 165.5. According to the results presented in Table 2C, private laboratories exhibited a statistically significant performance for selecting inappropriate agents compared to public laboratories. These findings were supported by the significant odds ratio of 1.8, a *p*-value of 0.001, and a χ^2 value of 68.1.

DISCUSSION

This study was the first to evaluate the appropriateness of AST and reporting practice in Yemen. The findings revealed significant gaps in knowledge and adherence to international standards. The findings highlight that testing and reporting practice for antimicrobial susceptibility were not conducted in accordance with international standards and exhibited high variability across different laboratories. Additionally, selective reporting of AST results was not being properly implemented. Although all laboratories participating in the study indicated that CLSI is the reference guidelines they rely on for AST, the majority (87.5%) rely on interpretive criteria provided by antimicrobial agents manufacturers to determine zone diameter or MIC breakpoints, rather than the CLSI guidelines. Reliance on interpretive criteria provided by antimicrobial agents manufacturers does not guarantee the continuous adjustment and updating of these interpretive criteria[35,36]. For testing and reporting *Enterobacteriaceae* susceptibility, the study identified low testing and reporting rates for primary appropriate agents, with significant variation between private and public laboratories. In private laboratories, reporting rates ranged from 13.9% for Tobramycin to 100% for Nitrofurantoin, with an overall rate of 45.3%. Public laboratories showed an overall utilization rate of only 31.2%, with some agents like Tobramycin, Fosfomycin, and Trimethoprim having a 0% reporting rate, while Nitrofurantoin was reported at 95.8%. The only other study with comparable findings on testing and reporting practice for *Enterobacteriaceae* susceptibility was a cohort study conducted across 48 laboratories in Ontario, Canada.

Table 2B: Selection of appropriate antimicrobial agents for testing but only selective reporting susceptibility of *Enterobacteriaceae* according to CLSI M100-2022.

Antimicrobial Agent	Lab. Ownership	Selected		Unselected		Total	Odds Ratio	95% C.I.		χ^2	*p*-value
		N	%	N	%			Lower	Upper		
Amikacin	Private	48	66.7	24	33.3	72	10.5	4.8	23.1	39.0	0.001
	Public	12	16.0	63	84.0	75					
	Total	60	40.8	87	59.2	147					
Ampicillin-Sulbactam	Private	34	47.2	38	52.8	72				46.1	0.001
	Public	0	0.0	75	100.0	75					
	Total	34	23.1	113	76.9	147					
Amoxicillin-Clavulanate	Private	64	88.9	8	11.1	72	0.8	0.3	2.4	0.1	0.722
	Public	68	90.7	7	9.3	75					
	Total	132	89.8	15	10.2	147					
Cefepime	Private	70	97.2	2	2.8	72	79.1	17.9	350.7	70.0	0.001
	Public	23	30.7	52	69.3	75					
	Total	93	63.3	54	36.7	147					
Cefotaxime	Private	68	94.4	4	5.6	72	7.1	2.3	21.7	14.3	0.001
	Public	53	70.7	22	29.3	75					
	Total	121	82.3	26	17.7	147					
Cefotetan	Private	2	2.8	70	97.2	72				2.1	0.146
	Public	0	0.0	75	100.0	75					
	Total	2	1.4	145	98.6	147					
Cefoxitin	Private	17	23.6	55	76.4	72				20.0	0.001
	Public	0	0.0	75	100.0	75					
	Total	17	11.6	130	88.4	147					
Ceftriaxone	Private	59	81.9	13	18.1	72	0.8	0.3	1.9	0.3	0.578
	Public	64	85.3	11	14.7	75					
	Total	123	83.7	24	16.3	147					
Cefuroxime	Private	58	80.6	14	19.4	72	5.9	2.8	12.4	23.7	0.001
	Public	31	41.3	44	58.7	75					
	Total	89	60.5	58	39.5	147					
Ciprofloxacin	Private	48	66.7	24	33.3	72	0.9	0.5	1.9	0.03	0.863
	Public	51	68.0	24	32.0	75					
	Total	99	67.3	48	32.7	147					
Doripenem	Private	17	23.6	55	76.4	72				20.0	0.001
	Public	0	0.0	75	100.0	75					
	Total	17	11.6	130	88.4	147					
Ertapenem	Private	38	52.8	34	47.2	72	15.6	5.7	43.3	37.7	0.001
	Public	5	6.7	70	93.3	75					
	Total	43	29.3	104	70.7	147					
Imipenem	Private	47	65.3	25	34.7	72	1.9	1.0	3.7	3.8	0.050
	Public	37	49.3	38	50.7	75					
	Total	84	57.1	63	42.9	147					
Levofloxacin	Private	63	87.5	9	12.5	72	6.1	2.7	14.1	20.4	0.001
	Public	40	53.3	35	46.7	75					
	Total	103	70.1	44	29.9	147					
Meropenem	Private	38	52.8	34	47.2	72	1.7	0.9	3.2	2.4	0.120
	Public	30	40.0	45	60.0	75					
	Total	68	46.3	79	53.7	147					
Piperacillin-Tazobactam	Private	59	81.9	13	18.1	72	7.6	3.6	16.3	30.3	0.001
	Public	28	37.3	47	62.7	75					
	Total	87	59.2	60	40.8	147					
Total	Private	730	63.4	422	36.6	1152	3.0	2.5	3.5	165.5	0.001
	Public	442	36.8	758	63.2	1200					
	Total	1172	49.8	1180	50.2	2352					

Table 2C: Inappropriate selection of antimicrobial agents for testing susceptibility of *Enterobacteriaceae* according to CLSI M100-2022.

	Lab. Ownership	Selected		Unselected		Total	Odds Ratio	95% C. I.		χ^2	*p*-value
		N	%	N	%			Lower	Upper		
Inappropriate agents for routine testing	Private	511	33.8	1001	66.2	1512					
	Public	345	22.0	1224	78.0	1569	1.8	1.5	2.1	53.5	0.001
	Total	856	27.8	2225	72.2	3081					
Unclassified agents	Private	70	7.6	857	92.4	927					
	Public	39	4.1	916	95.9	955	1.9	1.3	2.9	10.4	0.001
	Total	109	5.8	1773	94.2	1882					
Intrinsic resistant	Private	21	6.7	294	93.3	315					
	Public	6	1.6	366	98.4	372	4.4	1.7	10.9	11.5	0.001
	Total	27	3.9	660	96.1	687					
Not routinely reported on UTI isolates (Chloramphenicol)	Private	2	2.8	70	97.2	72					
	Public	2	2.7	73	97.3	75	1.0	0.2	7.2	0.0	0.967
	Total	4	2.7	143	97.3	147					
Total	Private	604	21.4	2222	78.6	2826					
	Public	392	13.2	2579	86.8	2971	1.8	1.6	2.1	68.1	0.001
	Total	996	17.2	4801	82.8	5797					

Among these primary appropriate agents, only the reporting rate of Nitrofurantoin on *Enterobacteriaceae*, was in agreement with Langford *et al.* observed reporting rate of Nitrofurantoin (96.7%) on *E. coli, K. pneumoniae*, and *P. mirabilis* urinary isolates[37].

For other key primary appropriate agents, such as Ampicillin, Trimethoprim-Sulfamethoxazole, and first-generation cephalosporins, Langford *et al.* study had documented higher reporting rates of 76.8%, 97%, and 96.2%, respectively. However, the current study found notably lower reporting rates for these agents among both private and public laboratories. In private laboratories, the reporting rates were 33.3% for Ampicillin, 73.6% for Trimethoprim-Sulfamethoxazole and 20.8% for first-generation cephalosporins. The situation was even more concerning in public laboratories, where the reporting rates were 32.7% for Ampicillin, 40.0% for Trimethoprim-Sulfamethoxazole and 2.7% for first-generation cephalosporins.

For secondary appropriate agents for testing but only selective reporting *Enterobacteriaceae* susceptibility, the present study demonstrated that both private and public laboratories documented higher rates of reporting for these agents than that of primary agents. In private laboratories, the overall reporting rate for secondary appropriate agents was 63.4% compared to 45.3% for primary agents. In public laboratories, the overall utilization rate of secondary appropriate agents was 36.8%, compared to 31.2% for primary agents. This practice is contrary to CLSI recommendations of cascade reporting, which promote the testing and reporting of primary agents in the vast majority of cases, while the reporting of secondary agents should be selective[38,39].

In private laboratories, the reporting rates for Amoxicillin-Clavulanic acid, third-generation cephalosporins, Cefepime, Levofloxacin, and Piperacillin-Tazobactam exceeded 80%. In contrast, public laboratories recorded similarly high reporting rates for Amoxicillin-Clavulanic acid and Ceftriaxone. These reporting rates were higher than those documented by Langford *et al*., which were 27.6% for Amoxicillin-Clavulanic acid and 30.1% for third-generation cephalosporins. Furthermore, Langford *et al*. study showed that Amoxicillin-Clavulanic acid was reported in only 1.2% of isolates that were susceptible to Ampicillin, while third-generation cephalosporins were documented in just 18.6% of urine isolates that were susceptible to first-generation cephalosporins[37].

The present study found that both private and public laboratories exhibited a high reliance on over-testing and over-reporting supplemental and alternative antimicrobial agents when testing susceptibility for uropathogen. This occurred despite the fact that the appropriate, recommended agents for routine testing and reporting were not being fully implemented as per CLSI guidelines. This observed practice directly contradicts the CLSI guidelines and recommendations, which do allow for the selective testing and reporting of some supplemental or alternative agents, but only in unusual cases when the full set of appropriate agents are not the optimal therapeutic options[39,40].

In the context of urine culture, this study highlighted a clear variation in the calculation of positive significant growth. Although all laboratories used 10-µl calibrated loops for plating samples, 75% considered the growth of 10 colonies (10^3 CFUs/ml) from clean-catch midstream specimens as the minimum colony count for a significantly positive UTI, while 25% considered the growth of 100 colonies (10^4 CFUs/ml) as significant. Additionally, about 63% of participating laboratories applied the same colony counts for determining positive significant growth from both clean-catch midstream samples and urine samples obtained through sterile procedures like suprapubic aspiration, without differentiation. This practice does not align with most international guidelines, which define a growth count greater than 10^5 CFUs/ml from clean-catch midstream

samples and greater than 10^2 CFUs/ml from urine samples obtained through sterile procedures as strong indicators of positive UTIs[34,41]. However, in certain cases, particularly in men, colony counts below 10^5 CFUs/ml, might be significant. In such instances, culture results should be interpreted alongside the patient's history, clinical symptoms, signs, and other laboratory findings[34, 41,42].

Regarding the preparation of inoculum suspension and matching it to the 0.5 McFarland standard, this step is consistently performed for all automated systems. However, for the manual disk diffusion method, this crucial step for providing consistent and reliable AST results, is not performed by the vast majority of laboratories (62.5%). This practice violates the recommendations of international guidelines[38]. Conversely, this study revealed that 75% of participating laboratories do not perform regular internal quality control tests using approved quality control strains. This practice contradicts international guidelines, which emphasize the importance of consistently assessing quality control to ensure the accuracy and reproducibility of AST results[38,43] .

CONCLUSIONS AND RECOMMENDATIONS

The study identified significant gaps in knowledge and adherence to international standards for AST and reporting practice. This lack of compliance with international standards contributes to variability and inconsistency across laboratories, affecting the quality of test results and leading to the over-reporting of inappropriate agents. Selective reporting of AST results is not being properly implemented. This practice can result in inappropriate treatment decisions and increased resistance. The study highlighted deficiencies in quality control practices, particularly in the preparation of inoculums suspensions and the lack of regular internal quality control using approved quality control strains. These practices are essential for ensuring the accuracy and reliability of AST results.

The finding of this study should draw the attention of health authorities in Yemen to develop and implement a national antimicrobial stewardship program, including a comprehensive guideline for AST and reporting across all laboratories. This guideline should be regularly updated to reflect the latest local susceptibility trends and adhere to international standards. A comprehensive and regular training and educational program should be implemented for laboratory personnel to enhance their knowledge. These programs should focus on the appropriate selection of antimicrobial agents, the accurate interpretation of culture yields and AST results, and emphasize the significance of selective reporting.

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AUTHOR'S CONTRIBUTION

Al-Khawi MA: conceived the idea, writing the manuscript, literature survey. **Bahaj SS:** supervision. **Al-Shami HZ:** supervision. **Al-Shamahy HA:** analysis, editing. **Al-Gunaid EA:** formal analysis. Final manuscript was checked and approved by all authors.

DATA AVAILABILITY

Data will be made available on request.

CONFLICT OF INTEREST

There is no conflict of interest associated with this study.

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