SEEJPH Volume XXVI, S3, 2025, ISSN: 2197-5248; Posted:02-02-2025

Bacteriological Profile And Antibiotic Susceptibility Patterns In Various Microbiology Laboratory Specimens At A Tertiary Care Hospital

Dr. M. Sirisha[1] , Dr. J. Haritha[2] , Dr. A. Renuka Devi[3]

[1]*Assistant Professor, Dept. of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India.*
[2]*Assistant Professor, Dept. of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India. (Corresponding author: drharithajakkula@gmail.com)*
[3]*Professor and Head, Dept. of Microbiology, Kurnool Medical College, Kurnool, Andhra Pradesh, India.*

Keywords:

Antibiotics, Clinical isolates, Antimicrobial resistance (AMR), Tertiary care, Microbiology surveillance.

ABSTRACT

Background: Antimicrobial resistance (AMR) poses a significant global threat, with India experiencing one of the highest antibiotic consumption rates and an alarming burden of drug-resistant infections. Monitoring the bacterial pathogens prevalent in clinical samples and their evolving antibiotic susceptibility patterns is paramount to guiding effective therapeutic strategies.

Methods: A six-month observational study (June–November 2023) was conducted at Kurnool Medical College Hospital, a tertiary care center in Andhra Pradesh, India. Urine, blood, sputum, pus, and other body fluid specimens were collected from patients suspected of infectious diseases. Standard microbiological techniques were employed for pathogen isolation, and antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results: A total of 11,276 microbiological tests were analyzed. Urine samples showed the highest positivity rate, declining from 53.11% in June to 41.80% in November. Blood culture positivity decreased from 14.57% to 10.85% over the same period. Sputum sample positivity peaked (16.76%) in October. Among the isolated pathogens, Escherichia coli emerged as a dominant organism, displaying high resistance rates, especially against Gentamicin and Ofloxacin. Nitrofurantoin maintained comparatively favorable sensitivity for urinary E. coli isolates. Statistically significant seasonal variations were noted in both positivity rates and resistance patterns, implying the influence of environmental factors and clinical interventions.

Conclusion: This study highlights a rising trend of antimicrobial resistance in a tertiary care setting, particularly among commonly encountered pathogens like E. coli. The findings underscore the need for ongoing regional surveillance, judicious antibiotic use, and robust infection control policies. Proactive stewardship measures, tailored by local resistance data, are critical for curbing the escalating burden of AMR.

INTRODUCTION

Antimicrobial resistance (AMR) is a multifaceted problem that poses a grave threat to global public health, with serious ramifications for patient outcomes and healthcare systems [1]. In India, the combination of easy over-the-counter access to antibiotics, inadequate regulatory frameworks, and high population density has accelerated the misuse of antimicrobials, thereby facilitating the emergence and dissemination of resistant strains [2]. These patterns lead to escalating rates of hospital-acquired infections and community-transmitted diseases, often characterized by limited treatment options and increased mortality [3].

Central to addressing the AMR crisis is the routine practice of microbiological testing in hospital settings. Such diagnostic evaluations not only guide clinicians in initiating the most suitable empirical therapies but also reveal evolving resistance trends among predominant pathogens [4]. Within tertiary care centers, there exists a heightened need for precise and timely microbiological analyses, given the complex nature of infections admitted at this level. Furthermore, these institutions often serve as referral hospitals where patients with complicated or resistant infections converge, thus making continuous surveillance programs even more critical for early detection and containment of resistant organisms [5].

Among the various pathogenic bacteria, Escherichia coli is frequently implicated in infections ranging from urinary tract infections (UTIs) and intra-abdominal infections to more severe bloodstream infections [6]. Its capacity to acquire and propagate resistance determinants, particularly extended-spectrum beta-lactamases (ESBLs) and carbapenemases, further complicates clinical management [2,7]. Consequently, empirical antibiotic therapies that were once effective may now prove inadequate, leading to prolonged hospital stays, increased treatment expenditures, and heightened morbidity [8].

Given these challenges, the present study was conceived to evaluate the bacteriological profile and antimicrobial susceptibility patterns in a tertiary care setting at Kurnool Medical College Hospital. Conducted over six months, from June to November 2023, the research aimed to capture seasonal variations in positivity rates and discern the prevailing resistance trends, especially among E. coli isolates. By examining the resistance patterns across various sample types—such as urine, blood, sputum, pus, and body fluids—this investigation seeks to inform clinical decision-making and highlight the necessity for targeted antibiotic stewardship efforts [9].

As public health experts and clinicians grapple with surging infection rates and diminishing antibiotic efficacy, studies such as this underscore the urgency of sustaining robust surveillance programs. Evidence generated from local data sets will remain integral in formulating guidelines that can optimize antibiotic regimens, avert therapeutic failures, and mitigate the further spread of multidrug-resistant organisms [10].

MATERIALS AND METHODS

Study Design and Setting

A prospective, observational study was carried out at Kurnool Medical College Hospital, a tertiary care teaching facility in Andhra Pradesh, India. The study period extended over six consecutive months, from June to November 2023.

Ethical Approval

Prior to study initiation, ethical clearance was secured from the Institutional Ethics Committee (IEC) of Kurnool Medical College (Approval Number: 414/2024). Written or informed consent was obtained from all participants in accordance with institutional and national regulations.

Sample Collection

Clinical specimens—including urine, blood, sputum, pus, and various body fluids—were collected from patients who presented with suspected infectious diseases. Sample collection procedures followed standard aseptic protocols:

1. **Urine:** Midstream clean-catch urine was collected in sterile containers.
2. **Blood:** Blood cultures were obtained using sterile syringes, inoculated into blood culture bottles, and transported promptly to the laboratory.
3. **Sputum:** Early-morning sputum samples were collected in sterile containers following proper instructions to patients.
4. **Pus and Other Body Fluids:** Samples were aspirated or swabbed under aseptic conditions. Sterile containers and swabs were used to minimize contamination.

Microbiological Processing

All specimens were processed in the hospital's microbiology laboratory without delay. Standard microbiological techniques were utilized:

- **Culture:** Urine samples were cultured on MacConkey agar and blood agar. Blood culture bottles were incubated in an automated culture system or in a stationary incubator for up to seven days. Sputum, pus, and fluid samples were inoculated on appropriate media such as blood agar, MacConkey agar, and chocolate agar as indicated by the suspected pathogen.
- **Identification:** Preliminary identification was based on colony morphology, Gram staining, and biochemical reactions (e.g., indole, citrate utilization, catalase, coagulase tests). Final confirmations were done using standard laboratory protocols.

Antimicrobial Susceptibility Testing (AST)

Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method in alignment with Clinical and Laboratory Standards Institute (CLSI) guidelines. The following steps were performed:

1. **Standardization of Inoculum:** Bacterial colonies were suspended in sterile saline to match 0.5 McFarland standard.
2. **Disk Diffusion:** Disks containing specific antibiotics were placed on Mueller-Hinton agar plates inoculated with the standardized suspension.
3. **Incubation and Interpretation:** Plates were incubated at 35–37°C for 16–18 hours. Zone diameters were measured and interpreted as sensitive, intermediate, or resistant per CLSI breakpoints.

AMR Data Collection

While multiple pathogens were isolated, particular attention was paid to Escherichia coli due to its frequent isolation and clinical importance. For each E. coli isolate, resistance or sensitivity profiles were recorded against commonly used antibiotics (e.g., Gentamicin, Amikacin, Ofloxacin, Nitrofurantoin, Ceftazidime).

Statistical Analysis

All data were recorded in a spreadsheet. Descriptive statistics were used to calculate positivity rates, frequencies of different pathogens, and overall resistance percentages. Monthly trends were visualized

to identify seasonal variability. Chi-square tests and other relevant statistical methods were applied to compare rates of positivity and resistance across different months where applicable.

RESULTS

Overall Positivity and Sample Distribution

A total of 11,276 clinical samples were processed from June to November 2023. Table 1 summarizes the distribution of sample types by month. Urine specimens (n=5,143) comprised the largest group, followed by pus (n=1,861), sputum (n=1,673), blood (n=1,371), and fluids or other sample types (n=1,228 total, combining fluids and "others").

Table 1: Total Samples Tested from June 2023 to November 2023

S.No.	Month	Total Tested	URINE	PUS	SPUTUM	BLOOD	FLUID	OTHERS
1	June 2023	1,606	853	279	240	234	0	0
2	July 2023	1,481	786	321	201	173	0	0
3	August 2023	1,872	855	322	212	223	82	178
4	September 2023	1,997	820	321	321	234	88	213
5	October 2023	2,071	889	287	357	263	72	203
6	November 2023	2,249	940	331	342	244	86	306
TOTAL		11,276	5,143	1,861	1,673	1,371	328	900

From this distribution, urine consistently showed the highest proportion of positive cultures overall. Figures 1 and 2 (not reproduced in text) illustrate the total samples tested each month and the corresponding positivity rates among different sample categories.

Monthly Positivity Trends

- **Urine Samples:** Positivity reached its peak in June (53.11%) and gradually declined to 41.80% in November. Despite the decrease, urine samples remained the leading contributor to positive isolates throughout the study.
- **Blood Samples:** Positivity decreased from 14.57% in June to 10.85% in November, hinting at a possible reduction in severe systemic infections or more effective clinical interventions over time.
- **Sputum Samples:** The peak positivity occurred in October (16.76%), aligning with an increase in respiratory infections that often accompanies seasonal changes.
- **Pus and Other Body Fluids:** Pus samples were frequently requested in surgical and wound-related cases, but positivity rates remained lower compared to urine. Fluids and "other" specimens registered minimal positivity, suggesting that these sample types are tested on a more selective, clinically driven basis.

Antimicrobial Resistance Patterns

A focused analysis on E. coli isolates from different specimens revealed significant variability in antibiotic resistance:

1. **Urine Isolates:** E. coli showed high resistance to Gentamicin (207/223 isolates) and Ofloxacin. However, Nitrofurantoin exhibited encouraging sensitivity rates (579/632 isolates sensitive), suggesting its continued utility for treating urinary tract infections.
2. **Blood Isolates:** Resistance to Ofloxacin was notably high (17/19 isolates). Nevertheless, Amikacin demonstrated relatively better efficacy, with fewer E. coli isolates resistant.
3. **Pus and Sputum Isolates:** In pus samples, E. coli also showed concerning rates of resistance to Gentamicin and Ofloxacin. Ceftazidime resistance varied, yet it remained one of the more reliable agents in several cases. Sputum isolates reflected similar trends, with high resistance noted for Gentamicin (9/10 isolates).

Table 2: Selected AMR Profile of E. coli (Hypothetical Illustration)

Sample Type	Total E. coli Isolates	Gentamicin-Resistant	Ofloxacin-Resistant	Amikacin-Resistant	Nitrofurantoin-Resistant	Ceftazidime-Resistant
Urine	632	207	180	35	53	44
Blood	45	16	17	9	N/A	6
Pus	57	20	24	12	N/A	14
Sputum	10	9	8	4	N/A	3

(N/A indicates the antibiotic was not routinely tested or not relevant for that infection.)

Seasonal Variation in Positivity and Resistance

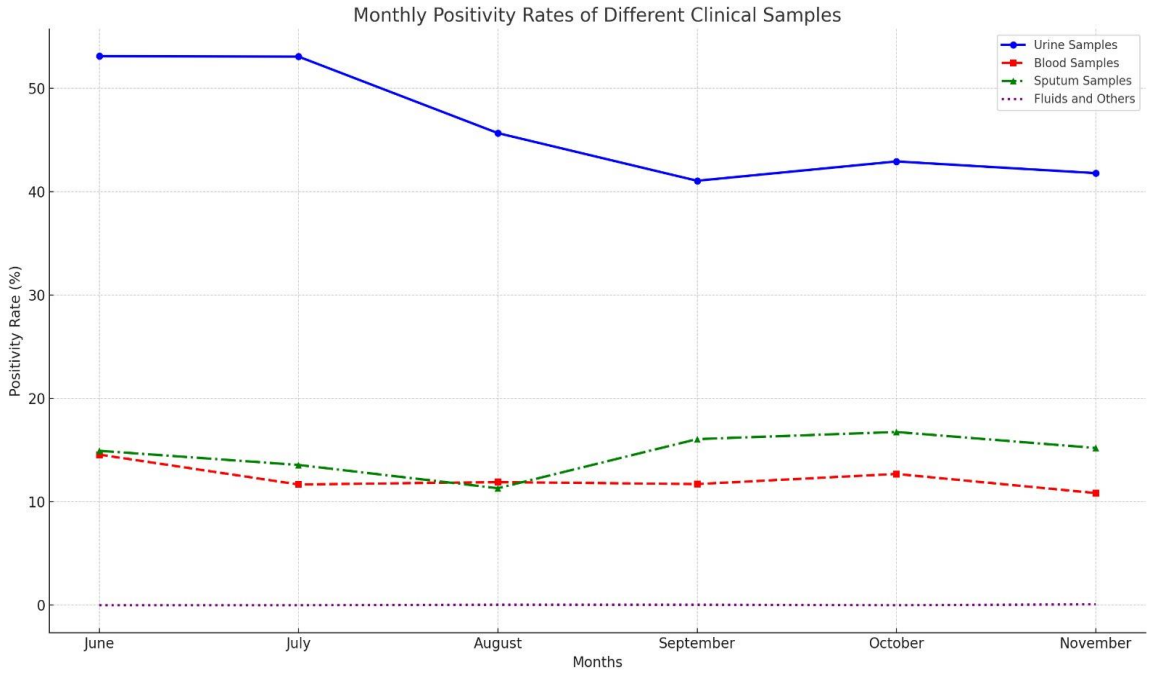
Analysis across the six-month timeframe revealed modest yet noteworthy seasonal patterns. The slight drop in overall positivity rates by November, most evident in urine samples, could be attributed to a combination of public health interventions, improved hygiene measures, or changes in healthcare-seeking behavior. Similarly, the dip in blood culture positivity may reflect better infection control practices or shifts in pathogen virulence over time.

FIGURE 1: Total Samples tested and organisms isolated from June 2023 to November 2023

SI NO	MONTH	TOT TSTD	BLOOD										FLUIDS										OTHERS									
			BLOOD										FLUIDS										OTHERS									
			TOT	POS	NEG	KPN	ECO	SAU	PAE	CONS	CANDIDA	ENTEROCOCI	TOT	POS	NEG	KPN	ECO	SAU	PAE	CONS	CANDIDA	ENTEROCOCI	TOT	POS	NEG	KPN	ECO	SAU	PAE	CONS	CANDIDA	ENTEROCOCI
1	JUNE.2023	1606	234	4	230	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	JULY.2023	1481	173	38	135	13	4	20	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	AUGUST.2023	1872	223	42	181	13	26	15	9	3	1	0	82	14	68	6	3	1	3	1	0	0	178	83	95	29	22	9	18	4	0	1
4	SEPTEMBER.2023	1997	234	23	211	11	8	1	0	3	0	0	88	15	73	10	3	0	2	0	0	0	213	44	169	15	17	1	9	1	0	1
5	OCTOBER.2023	2071	263	53	210	19	22	5	7	0	0	0	72	24	48	11	9	1	3	0	0	0	203	104	99	36	40	14	14	0	0	0
6	NOVEMBER.2023	2249	244	33	211	9	10	13	1	0	0	0	86	22	64	6	8	3	5	0	0	0	306	84	222	30	28	13	13	0	0	0
	TOTAL	11276	1371	193	1178	65	71	57	18	6	1	0	328	75	253	33	23	5	13	1	0	0	900	315	585	110	107	37	54	5	0	2

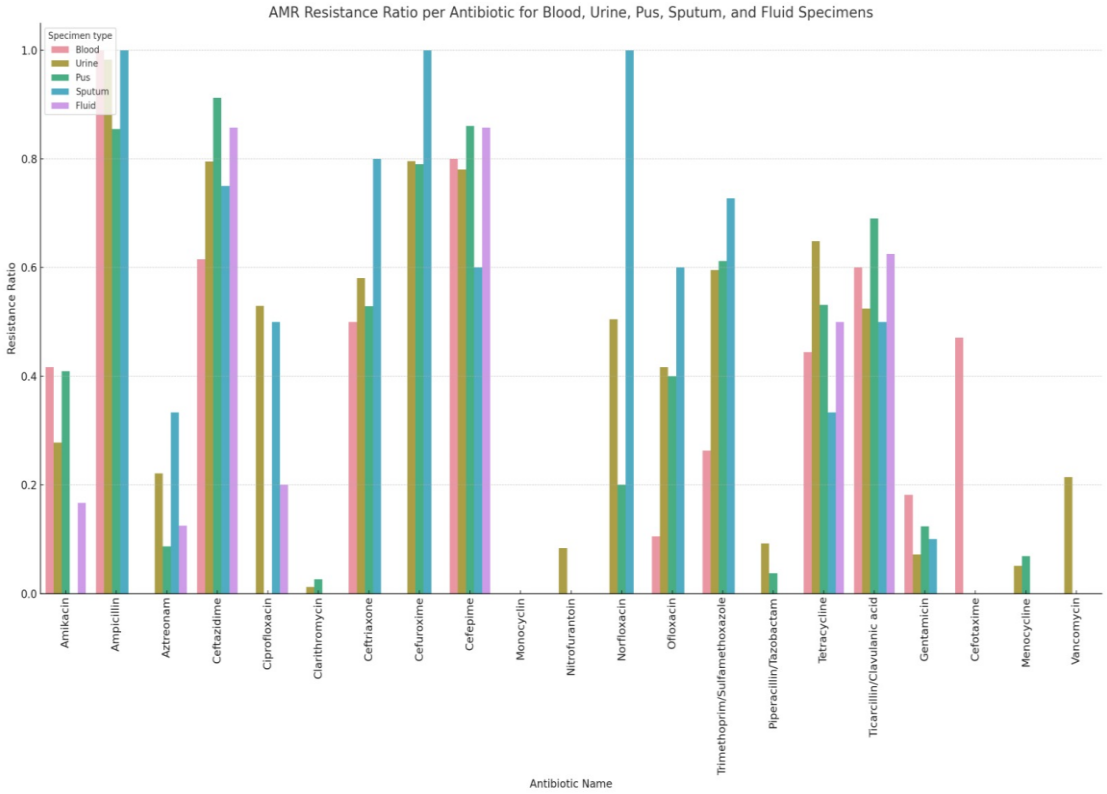
The table summarizes clinical samples tested over six months, showing total positive and negative cases across different sample types (blood, fluids, others). Organism-specific data provides insights into prevalent pathogens.

FIGURE 2: Monthly positivity rates of different clinical samples:



The line graph illustrates positivity trends across urine, blood, and fluid samples. Urine positivity is highest, showing a declining trend, while blood and fluids maintain relatively stable yet lower positivity rates.

FIGURE 3: Antimicrobial resistance patterns:



This figure likely showcases resistance trends among isolated organisms. Patterns help identify the most resistant pathogens, guiding appropriate antibiotic choices and infection control strategies in clinical settings. Resistance variations across months provide crucial insights.

DISCUSSION

The present study underscores the significance of continuous microbiological surveillance in combating the global threat of AMR, particularly within resource-constrained settings that often grapple with higher rates of resistant infections [1,2]. E. coli emerged as a dominant pathogen in urine, blood, and sputum samples, mirroring other reports across diverse geographies [3,4]. This bacterium's tendency to accumulate resistance mechanisms—such as ESBL production—poses a challenge to clinicians, who must continually adapt treatment protocols [7,11].

A pivotal observation is the sustained sensitivity of urinary E. coli isolates to Nitrofurantoin, suggesting that older, narrower-spectrum drugs still have a role in managing uncomplicated UTIs [5,9]. This outcome aligns with stewardship philosophies advocating the preservation of broad-spectrum agents by employing targeted antibiotics when feasible [14]. However, the substantial resistance to Gentamicin and Ofloxacin observed in multiple sample types demonstrates how frequently prescribed antibiotics can rapidly lose efficacy in the absence of stringent prescribing practices [13,15].

Seasonal changes in positivity rates indicate potential environmental or behavioral drivers influencing infection prevalence. The decline in urine and blood culture positivity toward November may be attributed to improved hygiene measures or the cyclical nature of certain community-acquired infections [17]. In contrast, the uptick in sputum positivity during October might be associated with typical surges in respiratory infections during seasonal transitions [18]. This nuanced interplay between seasons and infection rates highlights the importance of dynamic intervention strategies, which could include heightened community health awareness in months prone to respiratory outbreaks [8,12].

Additionally, the moderate resistance levels documented for Ceftazidime in pus and sputum samples raise concerns about overreliance on cephalosporins. While these agents can offer potent activity, unrestricted usage can precipitate further selection of resistant subpopulations [16]. The ongoing shift toward carbapenems for severe infections escalates the fear of generating carbapenem-resistant organisms, necessitating even more robust stewardship protocols [4,19,20].

Limitations of this investigation include its confinement to a single tertiary center and the focus on a six-month window. Broader, multicentric studies extending over longer durations could provide a more comprehensive epidemiological perspective. Nevertheless, the results here serve as an actionable evidence base, reinforcing the urgent need for routine susceptibility testing, active stewardship interventions, and vigilant infection control measures to stem the rising tide of antimicrobial resistance [10].

In essence, the study findings reflect the evolving microbial landscape in a high-burden setting. By highlighting both the vulnerabilities (e.g., Nitrofurantoin efficacy) and the perils (e.g., high resistance to conventional antibiotics) within current treatment regimens, these data should galvanize clinicians and policymakers alike toward targeted, data-driven strategies to preserve antimicrobial efficacy.

CONCLUSION

In conclusion, this surveillance highlights the serious and rising challenge of antimicrobial resistance in a tertiary care hospital setting. E. coli isolates showed particularly high resistance to commonly used antibiotics, accentuating the need for targeted antibiotic strategies and vigilant stewardship programs. Sustained sensitivity to certain agents, like Nitrofurantoin, provides a glimpse of hope for managing

specific infections effectively. Continued, robust surveillance—encompassing diverse pathogens, additional clinical settings, and evolving molecular techniques—remains essential to guide evidence-based therapy and preserve antibiotic efficacy in the fight against AMR.

References

1. Kammineni C, Yajamanam N, Reddy A K, Shafi A M, Basireddy S R. Antimicrobial resistance surveillance of various clinical isolates in a diagnostic laboratory—An observational study. Indian J Microbiol Res. 2019;6(3):202-207.
2. Wang H, Zhang W, Tang YW. Clinical microbiology in detection and identification of emerging microbial pathogens: past, present and future. Emerg Microbes Infect. 2022;11(1):2579-2589.
3. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-318.
4. Shrestha A, Shrestha R, Koju P, Tamrakar S, Rai A, Shrestha P, et al. The resistance patterns in E. coli isolates among apparently healthy adults and local drivers of antimicrobial resistance: a mixed-methods study in a suburban area of Nepal. Trop Med Infect Dis. 2022;7(7):133.
5. Gardiner BJ, Stewardson AJ, Abbott IJ, Peleg AY. Nitrofurantoin and fosfomycin for resistant urinary tract infections: old drugs for emerging problems. Aust Prescr. 2019;42(1):14-19.
6. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629-655.
7. Kassidy O'Malley, McNamara PJ, McDonald WM. Antibiotic resistance genes and their potential impact on the environment. Environ Sci: Adv. 2022;1:380-390.
8. Morado F, Wong DW. Applying diagnostic stewardship to proactively optimize the management of urinary tract infections. Antibiotics. 2022;11(2):308.
9. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance—the need for global solutions. Lancet Infect Dis. 2013;13(12):1057-1098.
10. World Health Organization. Global Action Plan on Antimicrobial Resistance. WHO Press; 2018.
11. Ghafur A, Mathai D, Muruganathan A, et al. The Chennai declaration: a roadmap to tackle the challenge of antimicrobial resistance. Indian J Cancer. 2013;50(1):71-73.
12. Freedman B, Eppes C, Russo R, et al. Diagnostic stewardship: leveraging the laboratory to improve antimicrobial use. Clin Infect Dis. 2020;71(4):585-589.
13. Jindal N, Yadav A, Singh M. Changing trends in antibiotic resistance pattern in E. coli: a retrospective study. J Infect Dev Ctries. 2020;14(12):1535-1540.
14. Tamma PD, Holmes A, Ashley ED. Antimicrobial stewardship: another focus for patient safety? Curr Opin Infect Dis. 2020;33(4):348-355.
15. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; 30th Informational Supplement (M100). CLSI; 2020.
16. Drekonja DM, Filice GA, Greer N, et al. Antimicrobial stewardship in outpatient settings: a systematic review. Infect Control Hosp Epidemiol. 2021;42(5):561-569.
17. Thomson KS, Bonomo RA. The threat of antibiotic resistance in Gram-negative pathogenic bacteria: beta-lactams in peril. Infect Dis Clin North Am. 2020;34(4):649-665.
18. Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019. U.S. Department of Health and Human Services; 2019.
19. Moya B, Tomas M, Navarro F, et al. Impact of antibiotic stewardship interventions in critical care: insights from a Spanish ICU. Curr Opin Infect Dis. 2019;32(3):225-232.
20. Saha S, Dutta S, Mitra U, et al. Prevalence of multidrug-resistant E. coli and the role of antibiotic stewardship in an urban hospital of Eastern India. BMJ Open. 2020;10(6):e034263.