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Clinical and Laboratory Profile of Urinary Tract Infections Associated with Extended Spectrum β-Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae*

Sulaiman Ali Al Yousef[1], Sabry Younis[1], Eman Farrag[1], Husseiny Sh. Moussa[2], Faten Sayed Bayoumi[3], and Ahmed Mohamed Ali[1]

[1]Department of Medical Laboratory Technology, College of Applied Medical Sciences, University of Hafr Al Batin, Hafr Al Batin, Kingdom of Saudi Arabia, [2]Faculty of Women for Art, Science and Education, Ain Shams University, and [3]Microbiology Department, Faculty of Pharmacy, MSA University, Cairo, Egypt

Abstract. ***Background.*** Urinary tract infection (UTI) is mainly due to invasion of the urethra, bladder or kidneys by pathogens. The emergence of extended spectrum β-lactamases (ESBL) is responsible for frequently observed empirical therapy failures. ***Objectives.*** To study the clinical and laboratory characteristics of UTIs caused by ESBL producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumonia* (*K. pneumonia*). ***Methods.*** A cross-sectional clinical and laboratory study was performed at King Khalid Hospital, Hafr Al Batin, Saudi Arabia between March 2014 to October 2015. A total of 908 urine samples from suspected UTI patients was collected. Samples were isolated on Cysteine Electrolyte-Deficient (CLED) agar. Positive cultures were identified and tested for antimicrobial susceptibility by MicroScan® WalkAway-96 SI System, and then ESBL was confirmed by double disc synergy test (DDST) and phenotypic confirmatory disc diffusion test (PCDDT). ***Results.*** A total of 680 samples (288 males and 392 females) were culture positive. 520 samples (76.5%)of *E. Coli* were found and 160 samples of *K. pneumonia* were identified (23.5%). ESBL testing showed 296 (218 *E. coli* and 78 *K. pneumonia*) samples of positive isolates. Non-ESBL isolates showed highest resistance to ampicillin followed by Mezocillin and Trimethoprim-Sulphamethoxazole-which are usually recommended as the initial treatment of UTI—while ESBL isolates showed resistance to third generation cephalosporin along with Ampicillin and Trimethoprim-Sulphamethoxazole. In this study, four significant risk factors for ESBL infection such as diabetes, recurrent UTI, previous use of antibiotics and previous hospitalization were found. ***Conclusion.*** Identifying the risk factors and antibiotic susceptibility patterns associated with ESBL producing *E. coli* and *K. pneumonia* is a useful guide for treatment strategy and control of ESBL UTI.

Key words: *E . coli*, *K. pneumonia*, ESBL, UTI.

Introduction

UTIs are defined by invasion of any part of the urinary tract by microorganisms. UTIs are the second most common community-acquired infections in clinical practice worldwide [1]. Although most cases have a good prognosis, UTIs can cause significant morbidity, prolonged hospital stay and certain complications [2,3]. Microorganisms responsible for UTI such as *E. coli* and *K. pneumonia* have the ability to produce ESBL in large quantities, making UTI difficult to treat [2]. In addition, ESBL infections can cause serious complications, especially in patients with functional structural anomalies of the urinary tract, patients who have undergone a kidney transplant, patients with polycystic kidneys, or diabetic patients [4].

Unfortunately, because of excessive application of antibiotics at different hospitals (especially in developing countries), antimicrobial resistance to uropathogenic bacteria across the globe is emerging [5,6]. The common misuse, underuse, or overuse, as well as neglected local community susceptibility profiles of these agents, invariably resulted in the emergence of multi-drug resistant (MDR) isolates among pathogenic bacteria [7,8].

Address correspondence to Ahmed Mohamed Ali, Department of Medical Laboratory Technology, College of Applied Medical Sciences, University of Hafr Al Batin, P.O. Box 1684, Hafr Al Batin, Kingdom of Saudi Arabia; e mail: ahmedelyamani22@gmail.com

Worldwide data shows that there is increasing resistance among UTI pathogens to conventional drugs. Resistance has emerged even to newer and more potent antimicrobial agents. The sensitivity of screening for ESBLs can vary depending on the type of antimicrobial agent tested [9,10]. In the research field (i.e. Saudi Arabia), the prevalence of ESBLs varies greatly in different regions. Prevalence rates range from 11% to 36% [11].

Antimicrobial resistance surveillance is necessary to determine the problem and to guide empirical selection of antimicrobial agents for treating infected patients. The aim of this study was to determine the current prevalence, clinical and laboratory profile of ESBL producing *E. coli* and *K. Pneumonia* infections among uropathogenes isolated from patients with suspected UTI at King Khalid Hospital, Saudi Arabia.

Materials and Methods

Study setting. This cross-sectional study was performed at King Khalid Hospital, Hafr Al-Batin, Saudi Arabia between March 2014 to October 2015.

Study participants. Inclusion criteria for this study were patients with suspected UTI. Exclusion criteria were patients who received antimicrobial therapy during two weeks prior to visiting the hospital and clinical symptoms or physical signs indicating any disease other than UTI. The control group was 45 healthy individuals who did not have signs or symptoms of UTI or any other disease and with no previous hospitalization or history of UTI during last year.

Clinical procedures.

Specimens collection and processing. Early morning mid-stream urine was collected in a sterile container. Then, urine samples were centrifuged and resulting precipitates were cultured on Cysteine Electrolyte-Deficient (CLED) agar and incubated at 37°C for 48 hrs. The pour plate method was used for viable bacterial count.

MicroScan analysis. The MicroScan® WalkAway-9 SISystem (Siemens Healthcare Diagnostics Inc. USA) was used in laboratory identification. Antibiotics susceptibility testing was performed with Neg/BP/Combo 30-B1017-306E combination panels (Siemens Healthcare Diagnostics Inc. USA). All procedures were performed according to the manufacturer's instructions. The integrated Lab-Pro® version 1.12, (Siemens Healthcare Diagnostics Inc. USA) which includes the alert expert system, uses growth in the presence of cefpodoxime (4 µg/ml) and ceftazidime (1 µg/ml) at concentrations recommended by the CLSI for ESBL screening [12], as primary indicators of possible ESBL production. Minimum inhibitory concentrations (MICs) obtained for ceftriaxone and cefotaxime are interpreted according to CLSI breakpoints [10], and results may also trigger rules which alert users to possible ESBL production. These results were considered a positive ESBL screening result for the purpose of our study.

ESBL Confirmatory Tests.

Double Disc Synergy Test (DDST). The isolated single colonies were inoculated in peptone wa¬ter at 37°C for 2-6 hours. The turbidity was adjusted to 0.5 McFarland standard and lawn culture was made on Mueller-Hinton agar (Oxoid Ltd, Bashingstone, Ham-pire, and UK) using a sterile swab. Augmentin disc (20/10 µg) was placed in the center of the plate. Both sides of the augmentin disc, a disc of cefotaxime (30 µg) and ceftazi¬dime (30 µg) (Oxoid Ltd, Bashingstone, Ham-pire, UK), were placed with center to center distance of 15 mm to the centrally placed disc. The plates were incubated at 37°C overnight. Extended spectrum ß-lactamases production was inferred when inhibition zone of the third generation cephalosporin disc was increased towards the Augmen¬tin disc.

Phenotypic Confirmatory Disc Diffusion Test (PCDDT). A lawn culture of the organism was made and thirdgeneration cephalosporins, ceftazidime (30 µg) discs and ceftazidime+clavulinicacid (30 µg+10µg) discs (Oxoid Ltd, Bashingstone, Ham-pire, UK) were placed 25 mm apart. An increase of ≥5mm in the zone of inhibition for ceftazidime + clavulinic acid compared to ceftazidime was confirmed as ESBL producers.

Ethical Approval. Written informed consent was obtained from participants or the parent or the legal guardian of participants less than 18 years old. The study was approved by the standing committee for research ethics on living creatures of the University of Dammam, membership No: HAP-05-D-003, National Committee of Bio Ethics (NCBE), KSA. Registration No: IORG0006803, Office for Human Research Protections (OHRP), USA.

Statistical analysis. The analysis was done using MedCalc® (MedCalc, Mariakerke, Belgium) statistical software. The qualitative data were shown in the form of number and percent¬age. Two by two tables are used to evaluate the relation between a possible risk factor (Exposure) and an outcome (Disease). The risk was estimated using Odds Ratio (OR), 95% confidence interval (CI 95%) and Fisher exact test. Statistical value of $p<0.05$ was considered to be significant.

Table 1. *Distribution of ESBL and no ESBL isolates according to demographic presentation of enrolled participants.*

Parameters	Total N=908	No Growth N=228	Growth N=680			
			E. Coli N=520		*K. penumoniae* N=160	
			ESBL* N=218	Non ESBL N=302	ESBL* N=78	None ESBL N=82
Gender						
Male	**356**	**68**	**70**	**145**	**34**	**39**
1- 20 years	60	12	0	36	9	3
21- 40 years	108	32	39	24	11	2
41- 60 year	188	24	31	85	14	34
Female	**552**	**160**	**148**	**157**	**44**	**43**
1-20 years	44	6	8	25	0	5
21- 40 years	304	94	68	85	37	20
41- 60 years	204	60	72	47	7	18

Table 2. *Distribution of ESBL and non ESBL isolates according to clinical presentation of enrolled patients.*

Parameters	Total N=908	No Growth N=228	Growth N=680				OR (95% CI)	*P* value
			E. Coli N=520		*K. penumoniae* N=160			
			ESBL* N=218	Non ESBL N=302	ESBL* N=78	None ESBL N=82		
Previous hospitalization (Before 6 months)								
Yes	540	99	130	200	47	64	0.68(0.49 - 0.93)	0.016
No	368	129	88	102	31	18		
Fever								
Yes	612	140	148	211	53	58	0.91(0.66 - 1.27)	0.58
No	296	88	70	91	25	24		
Abdominal pain								
Yes	530	89	130	180	47	64	0.79(0.58 - 1.07)	0.13
No	378	139	88	122	31	18		
Recurrent UTI								
Yes	516	112	171	152	65	16	2.93(2.03 - 4.22)	<0.0001
No	392	116	47	150	13	66		
Flank pain								
Yes	425	104	108	162	31	20	0.98(0.73 - 1.33)	0.91
No	483	124	110	140	47	62		
Previous use of Antibiotic (Before 3 months)								
Yes	37	8	23	5	0	1	5.3(2.13 - 13.21)	0.0003
No	871	220	195	297	78	81		
Diabetic								
Yes	620	168	160	197	70	25	2.54(1.8 - 3.57)	<0.0001
No	288	60	58	105	8	57		
Enuresis								
Yes	268	84	60	78	22	24	1.05(0.75 - 1.49)	0.74
No	640	144	158	224	56	58		

*ESBL presentation in this table is according to PCDDT results. OR Odds ratio , CI= Confidence Interval. *ESBL presentation in this table is according to PCDDT results.

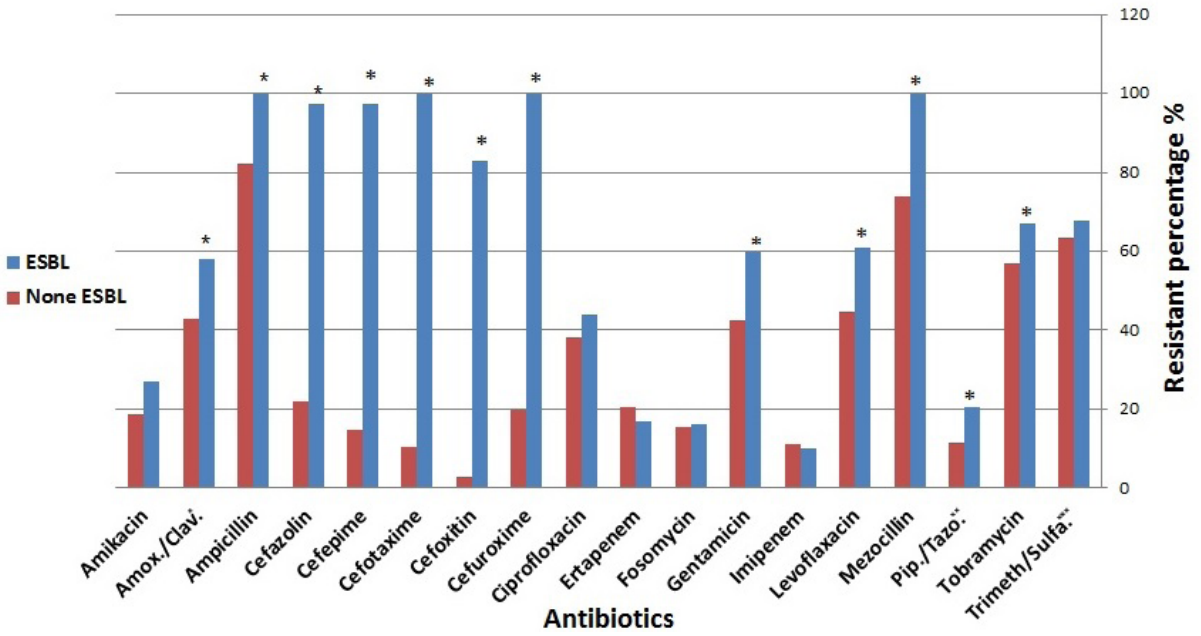

Figure 1. *Comparison of antimicrobial susceptibility between ESBL and non- ESBL producing isolates. (*statistically significant, P value was calculated from numbers of isolates not from percentage).*

Results

908urine samples were collected from patients with suspected UTIs (356 males and 552 females) at King Khalid Hospital during March 2014 to October 2015. Among 980 specimens, 680 (288 males and 392 females) were culture positive on CLED agar. Patient ages ranged from 12 months to 60 years. Demographic presentations of enrolled patients were shown in **Table 1**. All specimens of control group did not show any growth.

According to the results in **Table 2** and statistical analysis, ESBL infections by *E. coli* or *K. pneumonia* were more commonly found in diabetic patients (OR=2.54, p<0.0001), patients with recurrent UTI (OR=2.93 , p<0.0001), patients with previous antibiotic use before 3 months (OR=5.3, p=0.0003), and patients with previous hospitalization before 6 months (OR=0.68, p=0.016).

The most common isolated microorganisms were *E. coli* 520 (76.5%) followed by *K.pneumoniae*160 (23.5%). Antibiotic susceptibility test showed ful resistance of *K. penumonia* to Ampicillin, followed by Mezocillin (92.5%), Cefazolin (77.5%) and Cefuroxime (77.5%) and highest sensitivity to Imipenem (85%). *E. coli* showed high resistance to Ampicillin (86.9%), followed by Mezocillin (83%) and Trimethoprim/Sulfamethoxazole (66.15%). *E. coli* also showed high sensitivity to Fosomycin(90.7%), followed by Imipenem (90.7%) and Pipracillin/Tazobactam (86.92%). Antibiotics susceptibility results were shown in **Table 3**.

ESBL isolates showed overall higher resistance rate to antibiotics than non-ESBL ones, this increase was significant with third generation cephalosporines, Ampicllin, Amoxacillin/clavulinic, Mezocilline, Gentamycine, Levofloxacine, Topramycine and pipracilline/Tazobactam **(Figure 1)**.

680 resulted isolates, were screened for ESBL production using the MicroScan WalkAway-96 SI® System, according to CLSI guidelines.The results showed 232 (44.6%) *E. coli* and 88 (55%) *K. pneumonia* samples as positive. ESBL positive isolates were subjected to DDST and PCDDT ESBL confirmatory tests. The results showed 70 isolates out of the total (43.8%) were of *K. pneumonia* and 207 (39.8%) of *E. coli* were positive from DDST and 218 (41.9%) *E. coli* and 78 (48.8%) *K. pneumonia* were positive from PCDDT **(Figure 2)**.

Table 3. *Antibiogram of isolated K. pneumonia and E. coli.*

Antibiotic	No. of isolates(%)			
	Echerichia coli		*Klepsiella pneomnia*	
	Resistant	sensitive	Resistant	sensitive
Amikacin	36 (22.5)	124 (77.5)	116 (22.3)	404 (77.7)
Amox./Clav.*	88 (55)	72 (45.0)	248 (47.7)	272 (52.3)
Ampicillin	160 (100)	0 (0.00)	452 (86.92)	68 (13.08)
Cefazolin	124 (77.5)	36 (22.5)	248 (47.7)	272 (52.3)
Cefepime	120 (75)	40 (25.0)	224 (43.07)	296 (57.0)
Cefotaxime	112 (70.0)	48 (30.0)	224 (43.07)	296 (57.0)
Cefoxitin	76 (47.5)	84 (52.5)	180 (34.61)	340 (65.85)
Cefuroxime	124 (77.5)	36 (22.5)	248 (47.70)	272 (52.3)
Ciprofloxacin	96 (60.0)	64 (40.0)	180 34.46	340 (65.44)
Ertapenem	36 (22.5)	124 (77.5)	92 (17.70)	428 (82.30)
Fosomycin	60 (37.5)	100 (62.5)	48 (9.3)	472 (90.7)
Gentamicin	72 (45)	88 (55.0)	268 (51.54)	252 (48.46)
Imipenem	24 (15.0)	136 (85.0)	48 (9.3)	472 (90.7)
Levoflaxacin	60 (37.5)	100 (62.5)	272 (52.30)	248 (47.7)
Mezocillin	148 (92.5)	12 (7.50)	432 (83.00)	88 (17.0)
Pip./Tazo.**	36 (22.5)	124 (77.5)	68 (13.08)	452 (86.92)
Tobramycin	100 (62.5)	60 (37.5)	316 (60.77)	204 (39.23)
Trimeth/Sulfa.***	100 (62.5)	60 (37.5)	344 (66.15)	176 (33.85)

Amox./clav.=Amoxacillin/clavulinic; **Pip./Tazo.=Pipracillin/Tazobactam; ***Trimeth./Sulfa.=Trimethoprim/Sulfamethoxazole.

Discussion

UTI is one of the most common bacterial infections causing a significant amount of morbidity and mortality due to the invasion of urinary tract, often by pathogens belonging to the family *Enterobacteriacae*. In our study, isolated organisms were *E. coli* and *K. pneumonia. E. coli* was the most frequent occurring pathogen (76.5%) followed by *K. pneumonia* (23.5%). Similar results were reported by Kader et al.,. In Saudi Arabia, hey found that *E. coli* was the most commonly isolated pathogen from UTI patients followed by *K. pneumonia, Enterobacter* spp, and *P. aeruginosa* [13]. Also, many studies reported that *E. coli* was the most predominant pathogen[14-17].

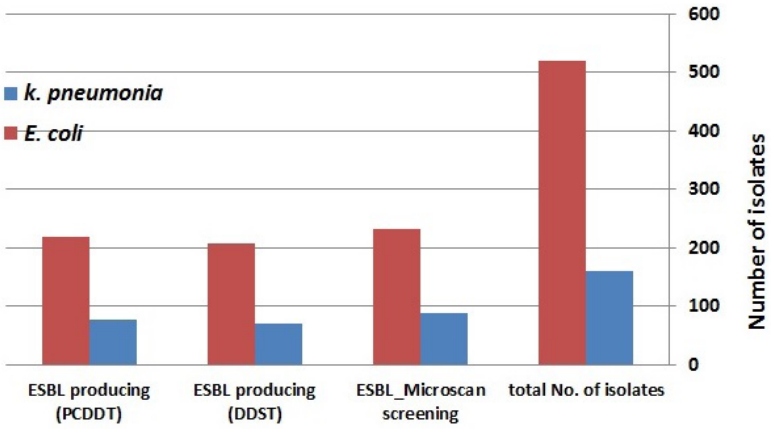

Figure 2. *ESBL pattern of isolated E. coli and K. pneumonia according to test type. (PCDDT=Phenotypic Confirmatory Disc Diffusion Test; DDST=Double Disc Synergy Test).*

In our study, compared with the previously reported results [21,23,24], the antimicrobial profile of ESBL and non ESBL isolates showed higher incidence of MDR *E coli* and *K. pneumonia*. Also, ESBL isolates showed significant increase in resistance to Amoxicilline/ Clavulinic, Ampicillin, Levoflaxacin, Gentamycine, Mezocillin, Tobramycine, pipracilline/Tazobactamand and third-generation cephalosporin compared to non-ESBL isolates. This resistance did not affect Fosomycin, Imipenem and Ertapenem **(Figure 1)**.

Periodic antimicrobial susceptibility surveillance in hospitals is an essential aspect of antimicrobial stewardship, as it prevents urosepsis, gives an idea about the most effective antibiotics that should be used in routine therapy, and possible emergence of multiple antibiotic resistant (MDR) strains of bacteria. In this study, *E. coli* showed the highest resistance to Ampicillin (86.92%), followed by Trimethoprim/Sulfamethoxazole (86%), and Mezocillin (83%). *E. coli* showed the highest sensitivity to Imipenem (90.7%). Similar results were obtained in a study taken place in Iran (18). Another study from United States reported that MDR *E. coli* showed 97.8% resistacet to Ampicillin, 92.8% to Trimethoprim- sulfamethoxazoleand, and 38.8% to Ciprofloxacin (19). Also, in our study, *K. pneumonia* showed ful resistance to Ampicillin, followed by Mezocillin (92.5%), and highest sensitivity to both Imipenem and Fosomycin (90.7%). Consistent results obtained by Okonkoet al also supported a high level of resistance to most of the antibiotics by *Klebsiella* spp [20].

Non ESBL isolates showed highest resistance to ampicillin, followed by Mezocilline and Trimethoprim-Sulphamethoxazole, which are usually recommended for initial treatment of UTI. ESBL isolates showed resistant to third generation cephalosporin along with Ampicillin and Trimethoprim-Sulphamethoxazole. Previous studies reported similar findings [21-23].

In our study, the prevalence rate of ESBL producing *E. coli* were 41.9% and *K. pneumonia* 48.7%. The overall prevalence of ESBL producers was found to vary greatly in different geographical areas and different institutes. A study in Saudi Arabia about ESBL producing *K. pneumonia* reported 66% [24]. Previous studies in India also have reported ESBL production varying from 28% to 84% [20]. The percentage is lowest in Maharashtra, reported by Rodrigues et al., [25]. In India, high prevalence of ESBL-producing Klebsiella pneumonia strains has been reported by various groups [23,26].

The present study demonstrates that the PCDDT was the most sensitive in detecting ESBL than DDST. DDST detected 43.8% in the case of *K. pneumonia* and 39.8% in the case of *E. coli* whereas PCDDT detected 41.9% of ESBL producers *K. pneumonia* and 48.8% of *E. coli.* Presence of ESBLs can be masked by the expression of AmpC β-lactamase, which can be generated by chromosomes of plasmid genes [27]. Out of the 384 clinical isolates of *K. pneumoniae*, 101 randomly selected isolates were screened for ESBL production by DDST and PCDDT. 79/101 isolates were found to be ESBL positive and 22 were negative [28].

In this study 4 significant risk factors were reported for ESBL infection: diabetes (p<0.0001), recurrent UTI (p<0.0001), previous use of antibiotics (p=0.0003), and previous hospitalization (p=0.016).

A retrospective study in Saudi Arabia reported recurrent UTI, hospitalization, surgical intervention and renal disease/renal transplant were the major risk factors for ESBL *E. coli* [29]. Another study in Saudi Arabia reported that mechanical ventilation, pervious hospitalization, previous use of antibiotics, and willing urinary catheter and previous ICU admission were the major risk factors for ESBL infection [30]. Use of antibiotics is an important risk factor for ESBL related infections [31,32]. Previous hospitalization and nosocomial ori¬gin of infections were significantly associated with ESBL acquisition, which is described by other au¬thors too. [31,33].

Study limitations. First, this study is a cross-sectional study and the small sample size may indicate that other, less prevalent risk factors for ESBL UTIs were not detected. Second, this study is concerned with only *E. coli* and *K. pneumonia* UTIs and other ESBL UTIs such as *pseudomonas aeruginosa* were not included, but they should be taken into account when observing ESBL UTIs. This study was performed in Hafr Al-Batin and ESBL incidence widely varies between geographical regions. Aulticenter evaluation is recommended to evaluate ESBL UTI problems in Saudi Arabia.

Conclusion. The present study clearly highlights that all the isolated ESBL producers were resistant to third generation cephalosporins but are still sensitive to carbapenems like Imipenem. There is an increase of resistance to a number of commonly used antibiotics to an alarming level. In the case of increasing ESBL incidence, ESBL testing for uropathogens along with conventional antimicrobial resistance would be useful as a treatment strategy and for controlling the spread of drug resistance. An important finding in this study is the determination of the risk factors for ESBL UTI caused by *E.coli* and *K. pneumonia*.

Moreover, drug resistance surveillance in hospital is necessary to know the impact of the drug resistance problem and for formation of a strict antibiotic policy for reducing the resistance level.

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