

*Research article***Analysis of urine culture isolates from seven laboratories in Western province of Sri Lanka****National Laboratory Based Surveillance- conducted by the Sri Lanka College of Microbiologists -2014**

SK Jayatilleke<sup>1</sup>, G Patabendige<sup>2</sup>, M Dassanayake<sup>3</sup>, GKD Karunaratne<sup>4</sup>, J Perera<sup>5</sup>, RRDP Perera<sup>6</sup>,  
WRPLI Wijesooriya<sup>6</sup>, NP Sunil Chandra<sup>6</sup>, J Kottahachchi<sup>7</sup>, D Athukorala<sup>7</sup>, T Dissanayake<sup>7</sup>

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**Abstract****Introduction:**

National Laboratory Based Surveillance of Antimicrobial Resistance in urinary isolates conducted by the Sri Lanka College of Microbiologists was started in 2011 in collaboration with the Ministry of Health of Sri Lanka.

**Methods:**

Pooled susceptibility data of urine culture isolates with a colony count of  $\geq 10^5$  CFU/ml from samples of non-catheterised patients received in 2014 were analysed using WHONET 5.6 software.

**Results:**

The majority of the isolates (3975/4979:79.8%) were Gram negative enteric organisms, commonly known as coliforms. The other bacterial isolates identified were *Enterococcus* spp. (254), *Pseudomonas* spp. (194), coagulase negative staphylococci (59), *Staphylococcus aureus* (36), *Acinetobacter* spp. (35) and Group B  $\beta$ -haemolytic streptococci (18).

The coliforms isolated from adults attending outpatient clinics (n=277) had 55.2% susceptibility to cephalexin and cephadrine, 54% to amoxicillin/clavulanic acid, 65.1% to nitrofurantoin,

<sup>1</sup> Sri Jayewardenapura General Hospital, Nugegoda, Sri Lanka

<sup>2</sup> National Hospital of Sri Lanka

<sup>3</sup> North Colombo Teaching Hospital, Ragama, Sri Lanka

<sup>4</sup> Lady Ridgeway Hospital for Children, Sri Lanka

<sup>5</sup> Department of Microbiology, Faculty of Medicine, University of Colombo, Sri Lanka

<sup>6</sup> Department of Microbiology, Faculty of Medicine, University of Kelaniya, Sri Lanka

<sup>7</sup> Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

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Address for correspondence: Dr. Kushlani Jayatilleka, Sri Jayewardenapura General Hospital, Nugegoda, Sri Lanka. Telephone +94714145056 Email: [kjayatilleke@gmail.com](mailto:kjayatilleke@gmail.com)

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48.3% to norfloxacin, 63.4 % to cefotaxime, 86.4% to gentamicin, 97.4% to imipenem and 100% to meropenem.

The isolates from adult hospitalized patients (n=1297) had 39.5% susceptibility to cefotaxime, 87.9% to meropenem, 62.6% to gentamicin and 31.9% to ciprofloxacin.

Coliforms isolated from paediatric outpatients (n=182) had 58.5% susceptibility to cephalexin and cephradine, 58.5% to amoxicillin/clavulanic acid, 80% to nitrofurantoin, 85% to cefotaxime, 86.5% to gentamicin and 89.7% to meropenem. Those from paediatric hospitalized patients (n=663) had 64.6% susceptibility to cefotaxime, 90.5% to meropenem and 80.2% to gentamicin.

### **Conclusion:**

Coliforms, the commonest category of organisms isolated had high resistance rate in hospitalized patients whereas the resistance was less in outpatients, especially in the paediatric age group.

*Keywords: Antibiotic resistance, Sri Lanka, urine isolates, coliform, Gram negative*

### **Introduction**

Surveillance of antibiotic resistance is important to decide the appropriate empirical antibiotic therapy for infections, guide policy recommendations and to assess the impact of resistance containment interventions. The National Laboratory Based Surveillance of Antimicrobial Resistance (NLBSA) conducted by the Sri Lanka College of Microbiologists was initiated following a meeting held at the Ministry of Health in 2011 to establish a national laboratory based surveillance of antimicrobial resistance. As an antibiotic resistance surveillance project was already ongoing for blood culture isolates, it was decided to collect data regarding the antibiotic susceptibility pattern of significant urine isolates and analyse this using WHONET software. WHONET is a software package recommended by the WHO for the management and analysis of microbiology laboratory test results with a focus on antimicrobial susceptibility test results.

### **Objectives**

1. To determine the aetiological agents of urinary tract infections with a colony count of  $\geq 10^5$  CFU/ml in mid-stream urine
2. To analyse the antimicrobial susceptibility patterns of urine culture isolates for the year 2014

### **Methodology**

Training in the use of WHONET was given to all Consultant Microbiologists in hospitals as well as Senior Lecturers in medical faculties of universities along with the data entry operating staff. Criteria for data fields, abbreviations and guidelines for antimicrobial panels were decided and distributed.

Midstream urine specimens were processed according to the standard protocol specified in the laboratory manual in microbiology.<sup>1</sup> Antibiotic susceptibility testing was performed according to the method routinely used in each centre which is either by the Clinical and Laboratory Standards Institute (CLSI) method<sup>2</sup> or by Stoke's comparative disk diffusion method.<sup>3</sup>

At the end of year 2014 all the microbiologists were requested to email the data folder of WHONET containing the data for the year 2014. Pooled susceptibility data of urine culture isolates with  $\geq 10^5$  CFU/ml from non-catheterised patients obtained from samples sent in 2014 were analysed using WHONET 5.6 software. Statistical analysis was carried out on resistance rates of coliform isolates from hospitalized patients (inpatients) and outpatient settings as well as from adult and paediatric populations for cephalexin, cefotaxime, amoxicillin/clavulanic acid and ciprofloxacin.

## Results

Data was received from seven centres. They were Sri Jayewardenapura General Hospital (SJGH) (1256 isolates from 1151 patients), The National Hospital of Sri Lanka (NHSL) (1260 isolates from 1132 patients), North Colombo Teaching Hospital, Ragama (CNTH) (1096 isolates from 1039 patients), Lady Ridgeway Hospital for Children (LRH) (929 isolates from 881 patients), Faculty of Medicine, Colombo (FMC) (65 isolates from 64 patients), Faculty of Medicine, Ragama (FMR) (19 isolates from unknown number of patients) and Faculty of Medicine, Sri Jayewardenapura (FMJ) (8 isolates from 8 patients).

The NHSL, SJGH, FMC, FMR and FMJ used the CLSI method while LRH and CNTH used Stokes method for susceptibility testing of urine culture isolates.

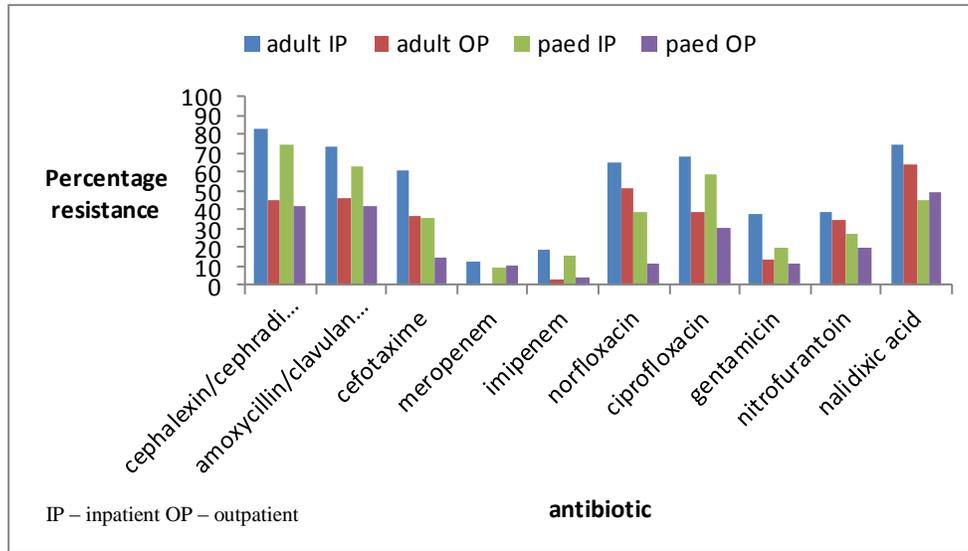
Of a total 4979 isolates, the majority were Gram negative enteric organisms (n=3975, 79.8%), commonly known as coliforms. The other bacterial isolates identified were *Enterococcus* spp. (254), *Pseudomonas* spp. (194), coagulase negative staphylococci (59), *Staphylococcus aureus* (36), *Acinetobacter* spp. (35) and Group B  $\beta$ -haemolytic streptococcus (18).

Table 1 and Figure 1 indicate the % resistance to the tested antibiotics and resistance in the different patient groups and locations.

**Table 1: Rates of antibiotic resistance in coliforms (pooled data)**

Antibiotic	Number tested	% resistance
ampicillin	1598	90.7
amoxicillin/ clavulanic acid	3702	49.3
cefotaxime	3049	52.9
meropenem	1654	9
nitrofurantoin	3044	30.4
nalidixic acid	3784	64.4
gentamicin	3776	29.3
norfloxacin	2977	59.3

Table 2 shows the % susceptibility of the isolates to tested antibiotics. The numbers tested for different antibiotics in different patient population differed, probably due to varying availability of antibiotic discs at different sites during different time periods. Non testing of some antibiotics in certain patient categories (eg. quinolones not tested in the paediatric population) and use of varying panels at the different test sites may also have contributed to the variation in isolate numbers tested. The analysis was not performed for antibiotics which were tested only in a small proportion of the population



**Figure 1: Antibiotic resistance rates of coliforms (pooled data) according to patient location**

**Table 2: Percentage susceptibility of urinary isolates to routinely tested antibiotics**

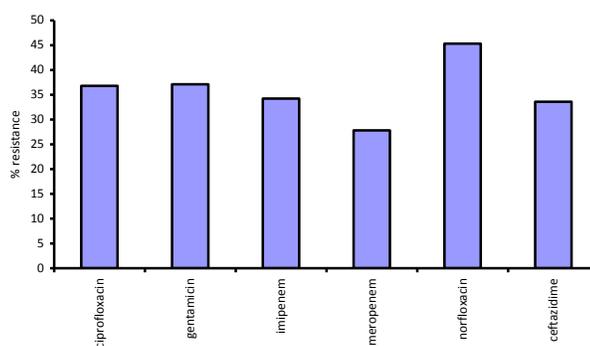
	Adult patients				Paediatric patients			
	Outpatient (OP) n=277		Inpatient (IP) n=1297		Outpatient n=182		Inpatient n=663	
	No. tested	% susceptible	No. tested	% susceptible	No. tested	% susceptible	No. tested	% susceptible
cephalexin/cephradine	203	55.2			118	58.5		
cefuroxime			2936	43.7				
amoxicillin/clavulanic acid	298	54	1223	34.1	130	58.5	77	57.1
nitrofurantoin	427	65.1			20	80		
norfloxacin	298	48.3						
ciprofloxacin			1281	31.9				
cefotaxime	298	63.4	1313	39.5	20	85	82	64.6
gentamicin	176	86.4	1298	62.6	186	86.5	81	80.2
imipenem	116	97.4						
meropenem	90	100	506	87.9	29	89.7	21	90.5

**Table 3: Comparison of percentage resistance in urinary isolates**

Antibiotic	Adult IP vs OP		Adult vs Paediatric	
	$\chi^2$	p value	$\chi^2$	p value
cephalexin	30.6	<0.0001	4.7	0.03
amoxicillin/clavulanic acid	61.6	<0.00001	21.1	<0.00001
ciprofloxacin	24.3	<0.00001	49.4	<0.00001
cefotaxime	44.4	<0.00001	30.0	<0.00001

IP – inpatient OP – outpatient

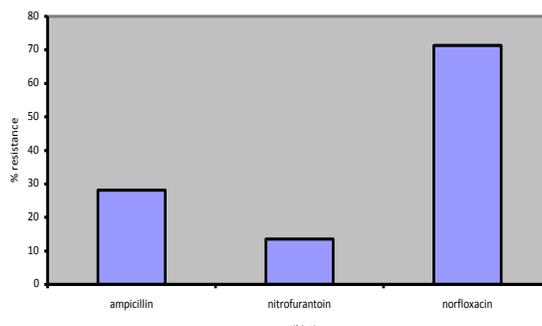
The resistance rates of coliforms for several antibiotics in hospitalized patients were significantly higher than in outpatients (Table 3). In addition, urinary isolates from adult patients were significantly more resistant than those from children (Table 3)



**Figure 2: Percentage resistance to different antibiotics of *Pseudomonas* species (pooled data)**

*Pseudomonas* spp. was isolated from 68 adult hospitalized patients, 33 hospitalized children, 6 adult outpatients and 10 paediatric outpatients. Percentage resistance to different antibiotics for *pseudomonas* species is shown in Figure 2.

*Enterococcus* species was isolated from 248 patients. Percentage resistance to different antibiotics is shown in Figure 3.



**Figure 3: Percentage resistance of *Enterococcus* spp.**

Sixteen (16) of 59 coagulase negative staphylococcal species were identified as *Staphylococcus saprophyticus*. Novobiocin discs were not available to identify *S. saprophyticus* in some centres and during certain time periods, and therefore some *S. saprophyticus* may have been reported as coagulase negative staphylococci. All isolates of *S. saprophyticus* were susceptible to nitrofurantoin and norfloxacin.

## Discussion

In the current study, coliforms were the predominant isolates comprising 79.8% of the total. In another study carried out in a tertiary care hospital in the Western province of Sri Lanka, 85.6% of isolates from hospitalized adults with features of urinary tract infections with a positive urine culture were coliforms.<sup>4</sup> The coliforms in the current study could not be identified up to species level due to unavailability of resources. It would be important to identify them to species level as different species have different mechanisms of resistance such as inducible resistance which may not be detected by disc diffusion methods of antibiotic susceptibility testing. Detection of Minimum Inhibitory Concentrations (MICs) for each antibiotic was not performed due to inadequate resources though recommended as better than disc diffusion methods of susceptibility testing.<sup>2</sup> Isolates could not be stored due to lack of storage space.

High resistance rates in coliforms were observed in hospitalized patients and resistance was significantly less in outpatients ( $P < 0.001$ ) for antibiotics analysed. This could either be due to hospital associated infections in the hospital population or because patients having infections with resistant organisms need hospitalization as they do not respond to outpatient treatment with oral antibiotics.

The resistance rates in coliforms from adult patients were significantly higher than in the isolates from the paediatric age group ( $P < 0.05$ ). This is possibly due to reduced exposure of children to antibiotics.

A relatively high proportion of urinary coliforms (74.9%) isolated from hospitalized adult patients in a Sri Lankan tertiary care hospital in 2009-2010 were susceptible to nitrofurantoin<sup>4</sup>, whereas in the present study only 61.7% were susceptible. In the 2009-2010 study, the susceptibility of coliforms to cefuroxime, co-amoxycylav, ciprofloxacin, norfloxacin, nalidixic acid, and gentamicin were 46.3%, 20.7%, 37.7%, 43.3%, 34.7% and 63.0% respectively, which is similar to the results obtained in the current study

The resistance rates of coliforms in the current study are high when compared to those observed in the United Kingdom.<sup>5</sup> Resistance rates reported of *E. coli* bacteraemia in England, Wales and Northern Ireland from 2009 to 2013 were much lower than the current study.<sup>5</sup> In contrast other South Asian countries such as India have reported higher rates of resistance to antibiotics.<sup>6</sup>

High resistance in coliforms were observed for orally available antibiotics such as nalidixic acid, fluoroquinolones, first generation cephalosporins and amoxicillin-clavulanic acid even in the outpatient setting with the exception of nitrofurantoin in paediatric patients,. This is suggestive of a substantial use of oral antibiotics in the community. The resistance rates reported in the

current study are much higher than rates in studies carried out in the outpatient setting of developed countries.<sup>7</sup>

Carbapenem resistance was high in *Pseudomonas* species. Enterococci remained susceptible to nitrofurantoin and ampicillin.

As all the laboratories with service of a specialist microbiologist in the state sector were eligible to participate in this surveillance, laboratories practicing different methods of antibiotic susceptibility testing participated in this study. This may have led to different interpretive criteria. Since these results were used to manage patients, and quality control procedures were in place and were satisfactory, all the data were considered as reliable.

Only urine cultures of patients who were not catheterized were considered for this study. However, as this was a laboratory based surveillance, patient details may not have been available all the time. This may be the reason for isolation of *Candida* spp. (8.2%) and coagulase negative staphylococci other than *S. saprophyticus* (43/4979, 0.86%) in this data pool, which are usually associated with catheter associated urinary tract infections.

## Conclusion

Coliforms are the commonest organism group causing urinary tract infections. High resistance rates in coliforms were observed in hospitalized patients compared with outpatients, especially in the paediatric age group. Resistance rates in coliforms were significantly higher in adults than in the paediatric population. High resistance rates in coliforms was observed for orally available antibiotics with the exception of nitrofurantoin, even in paediatric practice.

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