



## Antibiotic Resistance Patterns of *Escherichia Coli* Isolated from Different Laboratories Instruments

Saman Sana<sup>\*1</sup>, Muhammad Tahir Zakria<sup>2</sup>,  
Asmara Imtiaz<sup>2</sup>, Muhammad Hamza Sana<sup>2</sup>

<sup>1</sup>Department of Environmental Sciences, University of Veterinary and Animal Sciences, Lahore

<sup>2</sup>Department of Biology, Lahore Garrison University, Lahore

<sup>\*</sup>Corresponding Author's E-mail: [samansana@hotmail.com](mailto:samansana@hotmail.com)

### ABSTRACT:

*Antibiotics are being used to treat bacterial infections worldwide. Extensive and unchecked use of these antibiotics helps to develop resistance in microbes which is a growing issue for the medical specialists to treat different infections. To evaluate antibiotic resistance almost 125 samples were collected from different instruments of various institutional laboratories. Different microbes were isolated by using different culture media and further identified by biochemical characterization. Out of all isolated bacteria, only Escherichia coli (E. coli) were selected for antibiotic susceptibility. In the present study six antibiotics (colistin, norfloxacin, neomycin, chloramphenicol, nitrofurantoin and trimethoprim) were applied on the isolates of E. coli. 90% of the isolates of E. coli showed sensitivity or susceptibility towards chloramphenicol. It was concluded that chloramphenicol is the most effective antibiotic in treating the infections caused by E. coli.*

**Keywords:** *E. coli, antibiotic, antibiotic susceptibility, antibiotic resistance, Neomycin, Chloramphenicol*

### INTRODUCTION

Chemotherapeutic agents are those compounds or chemicals which can kill or inhibit the growth of

microbes (Dafale et al., 2016). For several decades many therapeutic agents have been widely used for the treatment of different diseases (Klotz and Schwab, 2005; Ray and Lahiri, 2009; Magrioti and

Kokotos, 2010). These chemotherapeutic agents have two types and may be available as synthetic or natural drugs. Antibiotics are chemicals that produced by microorganisms and inhibits the growth of bacteria. These are widely used to treat microbial contagions. The antibiotics kill or inhibit the growth of microbes in several ways such as by mutation in bacterial genome, rupturing cell membrane, production or enhancing specific type of protein which can kill or disrupt cell membrane (Piddock, 1996; Bengtsson and Wierup, 2006, Aminov, 2010). At the beginning, the use of these drugs was considered as a golden agent who can selectively kill the microbes or inhibit their growth and well-thought-out as an agent which does not produce any harmful effect on the host. Then by the advancement and further studies, it was noticed that microbes has ability to show resistance against antibiotics particularly effect the stage and time (Aminov, 2010). By inappropriate use of antibiotics, the chances of bacterial resistance are being enhanced. This is the reason why large amount and variety of antibiotics are required (Levy, 1998).

Millions of antibiotics have been produced and are in use from the last 60 years. Due to the augmented consumption and production of different antibiotics the severity infections are also increasing with the passage of time, leading to more expensive treatment.

On the basis of virulence

properties of enteric *E. coli* they are divided into six main groups such as enterotoxigenic, enteropathogenic, enteroinvasive, verotoxigenic, enterohaemorrhagic and enteroaggregative *E. coli* (Mandell et al., 2005; Biswas et al., 2006). According to Pitout, (2012) extra intestinal pathogenic *E. coli* resistance has been increasing against first line antibiotics since 2000 such as Cephalosporins and Fluoroquinolones. Van den Bogaard et al. (2001) studied antibiotic resistance patterns in *E. coli* against Ciprofloxacin and results revealed antibiotic resistance in *E. coli* is increasing. Present study was aimed to evaluate the susceptibility level of laboratory acquired microorganisms especially *E. coli* against various antibiotics. So that better treatment can be suggested for laboratory acquired infections of *E. coli*. Moreover lab worker's training is also required to stop spreading these infections among laboratory workers and community.

## MATERIALS AND METHODS

### Sampling Design and Sites

Seven swab samples were collected from different instruments including 2x centrifuges, incubator, 2x door handles, tap handles and bench tops) present in the laboratories of seven famous Universities of Lahore. These sites were Lahore Garrison University Lahore, University of Veterinary and Animal Sciences Lahore, Government Postgraduate College for Women Cooper Road Lahore, Government Postgraduate

College for Women Samanabad Lahore, Riphah University Lahore, Qarshi University Lahore and University of South Asia Lahore by direct swab method.

### Isolation of Microbes

Various bacterial isolates developed discrete colonies on nutrient agar. Pure culture was obtained from mixed culture using streak plate method (Geldreich and Rice, 1987) as shown in Fig. 1. Different colonies of nutrient agar were sub cultured, to obtain the specific active growth of bacteria for further biochemical analysis as shown in Fig. 2.

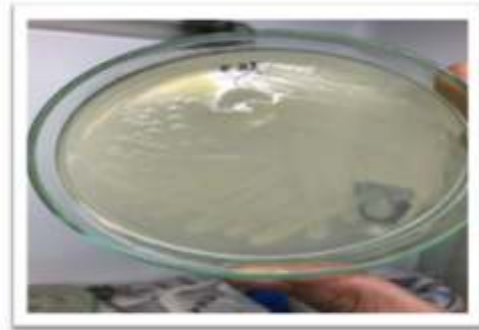


Fig. 1: Discrete Colonies Obtained by Streaking



Fig. 2: Subculture of bacterial isolates

### Differential and Selective Media

Different Selective media were used to isolate pathogenic strain of *E. coli* from other bacteria as shown in Table 1:

Table 1: Selective media used to isolate pathogenic strain of *E. coli*

Sr. No	Differential Culture Media	Pathogenic Organism
1	Mannitol salt agar	<i>Staphylococcus aureus</i>
2	Eosine methylene blue	<i>E. coli</i>
3	Macconky agar	<i>E. coli, Salmonella and Shigella</i>
4	Staph 110 media	<i>Staphylococci</i>
5	SS agar	<i>Salmonella and Shigella</i>

## Biochemical Characterization

Biochemical characterization of isolates (*E. coli*) was carried out using Gram Staining, Catalase test, Methyl Red test, VP test, Indole test, Citrate test, SIM Motility test / Hydrogen sulfide test, Nitrate Reduction test, Blood Agar, Urease test and Coagulase test by using methods of Cappuccino and Sherman, (2005).

## Determination of Antibiotic Susceptibility Using Disk Diffusion Method

For the determination of antibiotic sensitivity of various *E. coli* isolates Kirby Bauer disc diffusion method was used (Jacoby and Han, 1996). The inoculums were spread over Muller Hinton Plates by swabbing in three directions. Zone of inhibition of antibiotics were measured in millimeter using modified Kirby-Bauer method. Six antibiotics were used in

this study and named as Colistin, Norfloxacin, Neomycin, Chloramphenicol, Nitrofurantoin and Trimethoprim.

## Statistical Analysis

Results was evaluated using SPSS 21 (Statistical package for Social Science) software at 95% CI (confidence interval) and ( $P < 0.05$ ) was considered statistically significant.

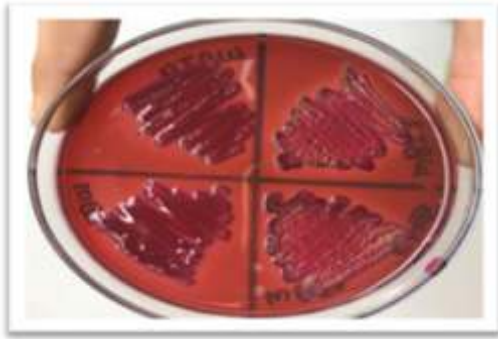
## RESULTS

### Biochemical Characterization & Selective Media

*E. coli* were isolated using biochemical characterization and growth on differential culture media. Biochemical test result and growth pattern of *E. coli* on differential culture media were mentioned in Table 2 and Fig 3.

**Table 2:** Result *E. coli* Biochemical Characterization and Differential Culture Media Growth

<b>Biochemical test</b>	<b>Result for <i>E. coli</i></b>
Catalase test	Positive
Coagulase test	Negative
Methyl red test	Positive
Urease test	Positive
SIM motility test	Positive
Indole test	Negative
Voges proskeur test	Positive
Nitrate reduction test	Negative
<b>Differential culture media</b>	<b>Results for <i>E. coli</i></b>
EMB (a)	Grow
Macconkey agar (b)	No growth
SS agar (c)	No growth
MSA (d)	Grow



EMB



Macconkey agar



SS agar



MSA

Fig. 3: Growth of *E.coli* strains on different agar media

### **Morphology of *E. coli***

Morphology and characterization of *E. coli* colonies was carried out and mentioned in Table 3

Table 3: Morphology and characterization of *E. coli* isolates

Name of Isolate	Size	Pigmentation	Form	Margin	Elevation		Optical Characteristics
					Flat	Opaque	
WBT-1	Large	Pale	Circular	Undulate	Flat	Opaque	Sediment
WBT-2	Moderate	Off-White	Irregular	Undulate	Convex	Opaque	Pellicle
WBT-3	Small	White	Irregular	Undulate	Raised	Opaque	Pellicle
WBT-4	Large	Pale	Circular	Serrate	Convex	Opaque	Flocculant growth
WBT-5	Large	Pale	Circular	Undulate	Flat	Opaque	Uniform fine turbidity
WBT-6	Large	Off-White	Circular	Entire	Convex	Opaque	Pellicle
poCA-WB	Large	Off-White	Circular	Entire	Flat	Opaque	Flocculant growth

### Antibiotic Sensitivity and Multi Drug Resistance

The antibiotic susceptibility and multi-drug resistance profiling for seven samples were done using disc diffusion method. (According to CLSI guidelines) Results were shown in Table 4 and 5, and Figure 4.

Table 4: Zone of Inhibition of Antibiotics

Antibiotics	Antibiotic Zone of Inhibition		
	Resistant	Intermediate	Susceptible
Trimethoprim	---	≤ 11-13mm	≤ 14mm
Neomycin	≤ 22mm	≤ 23-27mm	≤ 28mm
Nitrofurantoin	≤ 14mm	≤ 15-16mm	≤ 17mm
Norfloxacin	≤ 12mm	≤ 13-16mm	≤ 17mm
Cloremphenicol	≤ 12mm	≤ 13-17mm	≤ 18mm
Colistin	≤ 8mm	≤ 9-10mm	≤ 11mm

Table 5: Results of Antibiotic Resistance

Antibiotics	Total Sample Isolates	<i>E. coli</i> samples tested against various Antibiotics					
		Resistant	%age	Inter mediate	%age	Sensitive	Age
Trimethoprin	7	5	71.40%	0	0%	2	28.50%
Neomycin	7	7	100%	0	0%	0	0%
Nitrofurantoin	7	4	57%	1	14.20%	2	28.50%
Norfloxacin	7	4	57%	1	14.20%	2	28.50%
Cloremphenicol	7	2	29%	4	57%	1	14.20%
Clostin	7	5	71.40%	0	0%	2	28.50%

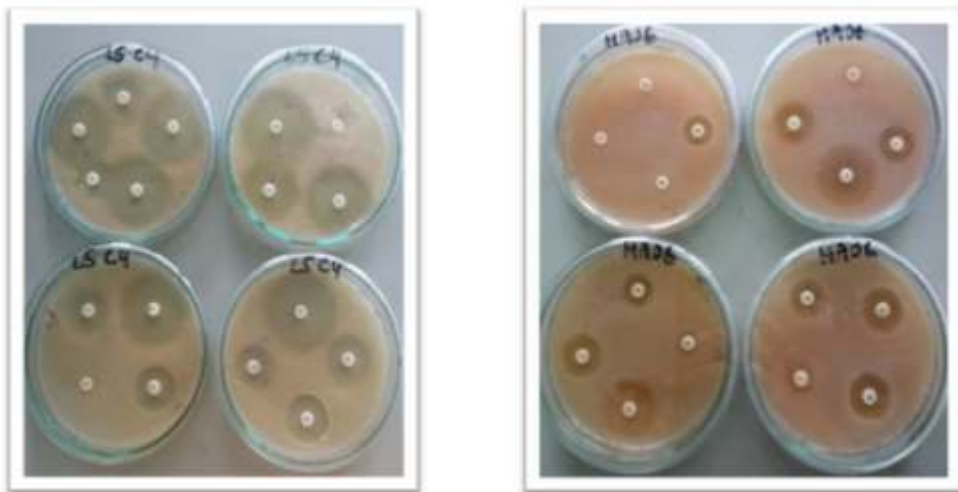


Fig. 4: Antibiotic Resistance and Susceptibility

Microbes were having mixed susceptibility towards antibiotics. Neomycin is least effective antibiotic against *E. coli* as no strain out of 7 x isolates was susceptible to it. Chloramphenicol was most effective antibiotic as maximum isolates were susceptible to this antibiotic. Results are highlighted in Fig. 5.



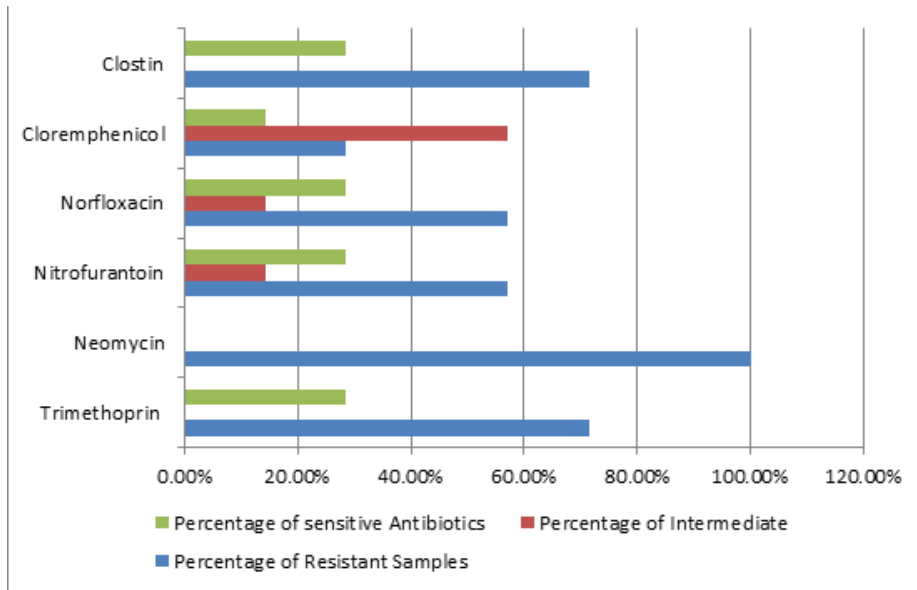


Fig. 5: Percentage of sensitivity to antibiotics

## DISCUSSION

LAI is of public health concern, as an infected worker may present a risk of transmission to his colleagues, relatives, family members or other citizens. LAIs are acquired through prolonged or continuous exposure to infectious agent in clinical microbiology laboratory. Pike, (1976) reported over 37 different species from the study that made on LAI. The Largest survey of LAIs was reported in 1976, there were 4079 LAIs cases in which 159 agents were involved. There are 173 deaths reported from LAIs (Iwao et al., 2012). This risk of LAI is mitigated by the application of safety guidelines issued by regulatory agencies and professional organizations. More than 43 % laboratory acquired infections (LAIs) are caused by bacteria. In our study all seven samples collected from different

institutions showed presence of different bacteria when grown on nutrient agar i.e. *S. aureus*, *E. coli*, *Salmonella* and *Shigella* and these microorganisms have 100% potential to act as infectious agents. In current study, we focused on susceptibility of *E. coli* for various antibiotics. Cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole are regularly practiced antibiotics to treat various infectious diseases caused by *E. coli* and resistance in microbes against these agents is responsible for delay in adopting an appropriate therapy (Bisson et al., 2002, Tumbarello et al., 2007). Some strains of *E. coli* possess pathogenic character due to the presence of virulent (Dho and Lafont, 1984) (Chulasiri and Suthienkul, 1989). In the present study following antibiotics was applied on the seven isolates of *E. coli* i.e. Colistin, Norfloxacin, Neomycin,

Chloramphenicol, Nitrofurantoin and Trimethoprim. Most of the isolates of *E.coli* showed resistance against Neomycin. Therefore it was almost ineffective against *E. coli*. 90% of the isolates of *E. coli* have shown susceptibility to chloramphenicol and it can be inferred that it is most effective one in treating the infections caused by *E. coli*. Previously a study was conducted in 2003 to check antibiotic sensitivity for *E.coli*. They reported that Piperacilin was most effective antibiotic and tetracycline was most ineffective antibiotic against *E.coli* because of isolates resistance against it. (Reinthal et al., 2003).

Earlier such studies have been conducted by foreigners but in Pakistan, it was conducted first study of its kind. The rationale behind this study was to sensitize the emerging issue of Laboratory acquired infections and transfer of resistant strains from laboratory workers to community. Therefore, sensitivity level of different isolates has been evaluated against various antibiotics. But the behavior of different isolates of different bacteria against antibiotics can vary in different areas, institutions or countries depending upon the type and level of exposure of microbes to the different antibiotics. This study also highlights the importance of safety protocols for lab workers to protect themselves from LAIs and will improve the health of community and lab workers.

## CONCLUSION

The untrained people acquire

LAIs as they don't follow standard operating procedures and biosafety measures for any microbial technique. They also spread these resistant and fatal bacteria to community. People also may become susceptible to infectious microbes by exposing to hazardous organisms and materials while visiting these institutes. Biotechnology and microbiology laboratories can minimize such incidents by formulating and adopting biosafety measures designed to protect their staff, the population and the environment. Laboratory staff training and education about epidemiology, pathogenicity and human susceptibility to the biological materials used in research is essential. Biological risk can be reduced and controlled by the correct application of internationally recognized procedures such as proper microbiological techniques, proper containment apparatus, adequate facilities, protective barriers and special training and education of laboratory workers. So, well trained people and fully developed infrastructure are needed to reduce LAIs.

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