

EFFICIENCY OF ANTBIOGRAM SENSITIVITY DETERMINATION AGAINST HUMAN PATHOGEN

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ABSTRACT

Antibiotic resistance pattern for human pathogen against different antibiotics was determined by the Kirby-Bauer method. The human associated pathogen were procured from Doctors Diagnostic Center, Trichy. Some commercial antibiotics are erythromycin (E¹⁰), ciprofloxacin (CIP¹⁵), polymyxin B (PB³⁰⁰), streptomycin (S²⁵), penicillin G (P¹⁰), chloramphenicol (C³⁰), gentamycin (GEN¹⁰), ampicillin A (A²⁵), itraconazole (IT¹⁰), rifampicin (RIF⁵), sulphamethizole (SM³⁰⁰) and sterile disc (as a negative control) against *B.cereus*, Coagulase negative *Staphylococcus*, *E.aerogenes*, *E.coli*, *Enterobacter* sp., *K.pneumoniae*, *P.aeruginosa*, *Proteus* sp., *Pseudomonas* sp., *S.aureus* and *S.typhi*. Ciprofloxacin, chloramphenicol, gentamycin and polymyxin antibiotics are more resistance activity against pathogen. In our results were compared the Clinical and Laboratory Standards Institute (CLSI) guides.

KEYWORDS: *Antibiotics, human pathogen, Muller Hinton agar*

INTRODUCTION

The word “antibiotic” refers to substances produced by microorganisms that act against another microorganism. Antibiotics do not include antimicrobial substances that are synthetic (sulfonamides and quinolones), or semisynthetic (methicillin and amoxicillin), or those which come from plants (quercetin and alkaloids) or animals (lysozyme). There are a large number of antimicrobial agents available for treating diseases caused by microorganisms. Such drugs are now an essential part of modern medical practice. The antimicrobial agents used in medical practice are aimed at eliminating the infecting microorganisms or at preventing the establishment of an infection. To be of therapeutic use, an antimicrobial agent must exhibit selective toxicity; it must exhibit greater toxicity to the infecting pathogens than to the host organism. A drug that kills the patient is of no use in treating infectious diseases, whether or not it also kills the pathogens. The new emerging pathogen with increasing antibiotic resistance, along with the susceptibility of immune compromised to common diseases has become an alarming problem worldwide. The introduction of a new class antibiotic that is efficacious and safe

which leads to wide spread use and thus development of resistance to treat many disease¹. A variety of antibiotic resistance strains were discovered by the work done^{2,3}.

An antibiotic should have the following characteristics:

- ❖ It should be toxic to the infecting organism while harmless to the host cells and the microbiota of the host.
- ❖ It should stay in toxic form for a sufficient amount of time to affect the infecting microorganism. If it changes to another form or is broken down in the body, it may not be useful.
- ❖ It should be sufficient in number to be able to reach the site of infection to kill the infecting agent.
- ❖ The infecting agent should be sensitive to it.

MATERIALS AND METHODS

Collection of clinical pathogen and antibiotics

The human normal flora was procured from Doctors Diagnostic Centre, Trichy. The Hi media antibiotics discs were purchased in Ponmani & Co. Chemical center, Trichy.



India map



Tamilnadu map

Determination of antibiotic susceptibility test⁴

The bacterial pathogens were grown on nutrient broth (NB) at 37°C for 24 hours incubation. A sterile cotton wool swab dipped into the 24 hours old bacterial suspension was spread evenly on the surface of the Muller Hinton agar plates. The inoculated plates were allowed to dry before placing the diffusion discs containing antibiotics. Susceptibility of the isolates to 12 types of antibiotics was performed using the standard Kirby-Bauer method. Commercially available discs (Hi-media) containing erythromycin (E¹⁰), ciprofloxacin (CIP¹⁵), polymyxin B (PB³⁰⁰), Streptomycin (S²⁵), penicillin G (P¹⁰), chloramphenicol (C³⁰), gentamycin (GEN¹⁰), ampicillin A (A²⁵), itraconazole (IT¹⁰), rifampicin (RIF⁵), sulphamethizole (SM³⁰⁰) and sterile disc (as a negative control) were placed on the surface of the MH agar plates and incubated at 30°C for 24 hours. The diameter of inhibition zones formed surrounding each isolate inclusive of diameter of the discs was measured. All isolates were tested duplicate for each type of antibiotic.⁵

RESULTS AND DISCUSSION

In the present study totally eleven species of human pathogens were tested against some commercial standard antibiotics disc. Effect of commercial antibiotics against some specific pathogen bacteria was analysed. Some antibiotics such as ciprofloxacin and polymyxin were sensitive antibiotics to all the pathogen. Likewise antibacterial activities of chloramphenicol and gentamycin were also observed. At similar

concentration, standard antibiotic, rifampicin showed 23mm of zone of inhibition against *S.aureus*, *P.aeruginosa* and *B.cereus*, but 22mm against *E.coli*. However, streptomycin showed highest zone of inhibition against *S.aereus*, (32mm) but lowest against *B.cereus* (27mm) at similar concentrations⁶. Onget *al.*⁷ evaluated that strongly susceptible to five types of antibiotics which include amikacin (AK³⁰), kanamycin (K³⁰), gentamycin (CN¹⁰), norfloxacin (NOR¹⁰) and tetracycline (TE³⁰). 20% of the isolates were resistant to penicillin G (P¹⁰), ampicillin (AMP¹⁰), streptomycin (S¹⁰), chloramphenicol (C³⁰), nitrofurantoin (F³⁰⁰), sulphanethoxazole (RL¹⁰⁰) and trimethoprim (W⁵). Similarly in the present investigation the effect of ciprofloxacin antibiotics has been well documented in the excellent zone formation as 13, 25, 20, 08, 27, 32, 30, 07, 15, 40 and 30 mm with *B.cereus*, Coagulase negative *Staphylococcus*, *E.aerogenes*, *E.coli*, *Enterobacter* sp., *K.pneumoniae*, *P.aeruginosa*, *Proteussp*, *Pseudomonas* sp., *S.aureus* and *S.typhi*. whereas chloramphenicol antibiotics also showed very good activity against *B.cereus* (30 mm), Coagulase negative *Staphylococcus* (30 mm), *E.coli* (15 mm), *Enterobacter* sp. (30 mm), *P.aeruginosa* (10 mm), *Proteus* sp. (30 mm), *Pseudomonas* sp. (15 mm), *S.aureus* (30 mm) and *S.typhi* (10 mm) zone of inhibition was measured. While the effect of gentamycin with moderate among the selected bacterium as 5, 27, 8, 15, 15, 4, 9, 25 and 20 mm zone of inhibition with *B.cereus*, Coagulase negative *Staphylococcus*, *E.aerogenes*, *E.coli*, *Enterobacter* sp., *P.aeruginosa*, *Pseudomonas* sp., *S.aureus* and *S.typhi* was recorded respectively. The polymyxin antibiotics was *B.cereus* (4 mm),

Coagulase negative *Staphylococcus* (7 mm), *E.aerogenes* (5 mm), *E.coli* (3 mm), *Enterobacter* sp. (7 mm), *K.pneumoniae* (10 mm), *P.aeruginosa* (5mm), *Proteus* sp. (4 mm), *Pseudomonas* sp. (5 mm), *S.aureus* (3 mm) and *S.typhi* (5 mm) recorded respectively when compared to sterile disc. Some of the other antibiotics also showed linear activity, which was observed by the *invitro* methods (Table 1; Fig. 1 and Plate I). *S. aureus* is a persistent nosocomial and community acquired pathogen. It became a global health concern, due to remarkable capability of developing drug resistant mechanism against most antimicrobial agents⁸. Generally, bactericidal compounds are more potent than bacteriostatic compounds. It is well established that bactericidal activity is an important determinant of clinical outcome⁹. The isolates were 100% resistant to gentamycin, streptomycin, tetracycline, and

chloramphenicol. Less resistance (90.9%) was shown to ofloxacin, saprofloxin and amoxycillin used. The minimum resistance was shown to ciprofloxacin (63.6%) and pefloxacin (54.5%)¹⁰. These antibiotics seem effective against the isolated *E. coli*. This corroborates with the work done¹¹ on antibiotics susceptibility pattern of *Escherichia coli* isolated from well water in Afikpo south eastern Nigeria. Additionally,^{12,13,14} have all previously reported bacterial resistance against ampicillin, gentamycin, erythromycin, tetracycline and ciprofloxacin at different times. The reason why some of these *E. coli* isolates showed high level of resistance to the antimicrobial agents used is an indication that these antibiotics have been abused, hence the possibility that they have acquired resistance

Table 1
Antbiotics sensitivity test against human pathogen

S.No	Name of the bacteria	Zone of inhibition (mm)											
		SM ³⁰⁰	IT ¹⁰	RIF ⁵	E ¹⁰	PB ³⁰⁰	CIP ¹⁵	S ²⁵	P ¹⁰	C ³⁰	A ²⁵	GEN ¹⁰	SD
1	<i>B.cereus</i>	-	-	-	-	4	13	-	-	30	-	5	-
2	Coagulase negative <i>Staphylococcus</i>	11	-	9	12	7	25	-	2	30	-	27	-
3	<i>E.aerogenes</i>	-	-	-	-	5	20	-	-	-	-	8	-
4	<i>E.coli</i>	10	3	6	10	3	8	4	-	15	-	15	-
5	<i>Enterobacter</i> sp	25	-	10	-	7	27	-	6	30	5	15	-
6	<i>K.pneumoniae</i>	-	-	3	-	10	32	10	-	-	-	-	-
7	<i>P.aeruginosa</i>	-	-	4	-	5	30	14	-	10	-	4	-
8	<i>Proteus</i> sp	28	-	20	15	4	7	7	-	30	-	-	-
9	<i>Pseudomonas</i> sp.	20	-	15	6	5	15	2	-	15	-	9	-
10	<i>S.aureus</i>	40	-	40	35	3	40	25	10	30	-	25	-
11	<i>S.typhi</i>	15	-	10	7	5	30	-	5	10	22	20	-

SM³⁰⁰-sulphamethizole, IT¹⁰-itraconozole, RIF⁵-rifampicin, E¹⁰- erythromycin, PB³⁰⁰-polymyxin B, CIP¹⁵- ciprofloxacin, S²⁵- Streptomycin, P¹⁰-penicillin G, C³⁰-chloramphenicol, A²⁵-ampicillin A, GEN¹⁰-gentamycin, SD -sterile disc

≤15 resistance, 16-20 intermediate, ≥21 sensitive

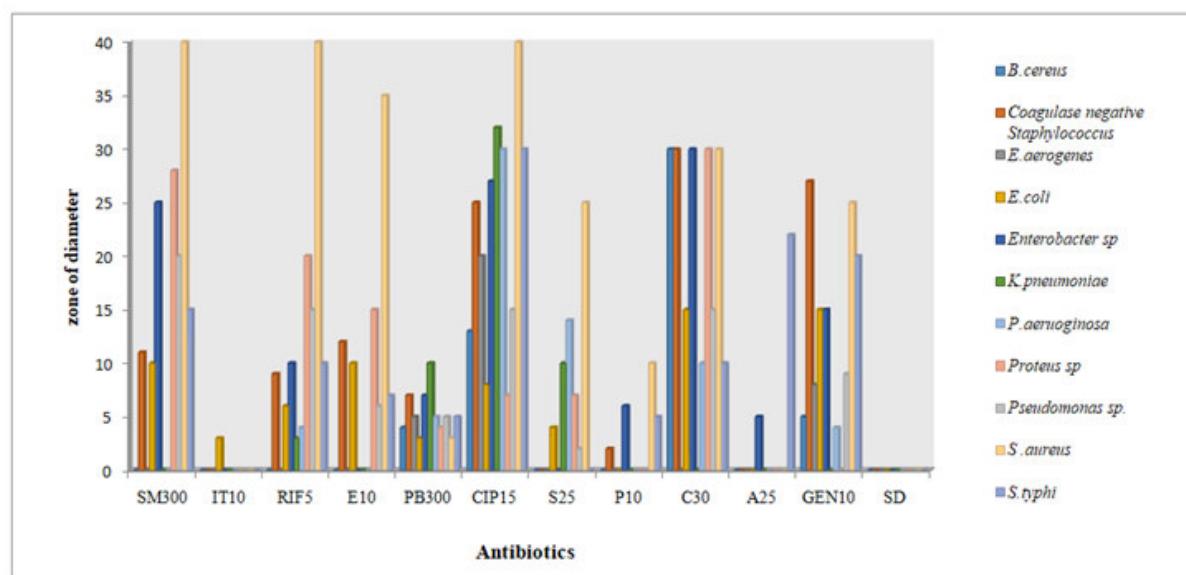


Figure 1
Sensitivity pattern of Antibiotics against human pathogen



Plate I
*Antibiotic susceptibility activity of *S. aureus**

CONCLUSION

It is concluded that the our study, the human flora are some occasional to change the pathogen to casuse disease in human beings. Some favourable condition *S. aureus* and *E. coli* are caused disease. In these times the commercial antibiotics are useful for human beings.

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

- Christopher W., Antibiotics Actions, Origins, Resistance. U.S.A: ASM Press; 2003. p. 345.
- Alvero C Antibiotic resistance of heterotrophic bacterial flora of two lakes. *Syst. Appl. Microbiol.* 19879: 169-172.
- Lobova TI, Maksimova EY, Popova LY, Pechurkin NS. Geographical and seasonal distribution of multiple antibiotic resistance of heterotrophic bacteria of Lake Shira. *Aquatic Ecol.* 2002 36: 299-230.
- Robert AP, Lorraine F, Walter M, Ronald MR. Laboratory exercises in Microbiology (3rd ed.). U.S: John Wiley & Sons, Inc. 2009.
- Liasi SA, Azmi TI, Hassan MD, Shuhaimi M, Rosfariza M, Ariff AB. Antimicrobial activity and antibiotic sensitivity of three isolates of lactic acid bacteria from fermented fish

product. *Budu. Malaysian J. Microbiol*; 2009 5(1): 33-37.

6. Pudi N, Gayatri DV, Anil KB, Murali MG, Seema K, Ramarao M. Studies on optimization of growth parameters for enhanced production of antibiotic alkaloids by isolated marine actinomycetes. *Journal of Applied Pharmaceutical Science*. 2016 6 (10): 181-188.

7. Ong KS, Chin HS, Teo KC. Screening of antibiotic sensitivity, antibacterial and enzymatic activities of microbes isolated from ex-tin mining lake. *African Journal of Microbiology Research*. 2011 5(17): 2460-2466.

8. Montefiore D, Rotimi VO, Adeymi-Doro FAB. The problem of bacterial resistance to antibiotics among strains isolated from hospital patients in Lagos and Ibadan, Nigeria. *J Antimicrob Chemother*; 1989 4:641-651.

9. Jianbo Xiao. Phytochemicals in food and nutrition. *Crit Rev Food Sci Nutr*; Epub ahead of print. 2015. p.

10. Joseph OA, Oluwaseun O OA, Nihinlola A, Alexander O. and Arwa S. Antibiotic susceptibility pattern of *Escherichia coli* Isolates from Clinical Sources at tertiary Health Care Settings, Ile Ife, South Western Nigeria. *Euro.J. of Experi. Biol*. 2017 7 :1:5.

11. Onuoha SC. Antibiotic susceptibility pattern of *Escherichia coli* Isolated from Well Water in Afikpo South Eastern Nigeria. AASCIT *Journal of Biology*; 2015 1: 38-42.

12. Mydrk ZZ. Antibiotics resistance among bacteria inhabiting surface and subsurface water layers in estuarines Lake Gardno. *Polish Journal of Environmental Studies*; 2002 11: 401-406.

13. Toth M, Smith C, Frase H, Mobashery S, Vakulenko S. An antibiotics resistance enzyme from a deep-sea bacterium. *J Am ChemSoc*; 2010 132: 816-823.

14. Cox G, Wright GD. Intrinsic antibiotics resistance: mechanisms, origin, challenges and solutions. *Int J Med Microbiol*; 2013 303: 287-292.