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Research Article

ANTIBIOGRAM OF SOME SELECTED SPECIES OF GRAM NEGATIVE BACTERIA ISOLATED FROM HOSPITAL ENVIRONMENT

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ABSTRACT
The antibiotic resistance is widely distributed among the strains found in wild. The introduction of a new antimicrobial agent is always followed by the emergence of resistance strains. In this study various classes of antibiotics were tested against the species of family enterobacteriaceae isolated from air and sewage of hospital associated environment. The multi drug resistance patterns of some selected species of the family enterobacteriaceae isolated from hospital associated environments were found out. The *enterobacter* spp. showed resistance towards the six classes of antibiotics and *Klebsiella* spp. and S*almonella* spp. towards five classes of antibiotics.

Key Word*: Multi Drug Resistance, Antibiotics, Enterobacteriaceae*

INTRODUCTION
The antibiotic resistance is widely distributed among the strains found in wild. The introduction of a new antimicrobial agent is always followed by the emergence of resistance strains. Previous studies prove that the emergences of resistance toward common antimicrobial compounds are always associated with consumption of these drugs en mass and inappropriate ways. Emergence of extended-spectrum b-lactamase (ESBL) producing Enterobacteriaceae (Meyer *et al*., 2010), carbapenemase-producing Gram negative bacteria (Furtado *et al*., 2010), multiresistant *Pseudomonas aeruginosa* (Weng *et al*., 2011) and *Acinetobacter baumannii* (Meyer *et al*., 2009) are some of the example of inappropriate uses of drugs.
Clinically significant Gram negative bacteria are and especially the members of the family Enterobacteriaceae acquired resistance towards antibiotics through the process of conjugation. Resistance factors are small independent extrachromosomal DNA molecules conferring resistance on one or more antibiotics to the bacteria which harbours them. Most of the resistant genes carry on conjugative elements, the resistance gene captured in mobile elements is due to random and illegitimate recombination events and there successful spread is achieved through appropriate selection pressure, the integron, characterised as transposons and plasmids conveying multiple antibiotic resistance determinants (Moartinez and Cruz, 1990., Ouellette *et al*., 1987., Stokes *et al*., 2001., Sundstrom *et al*., 1988).
Integrons play a leading role in the acquisition and spread of antibiotic resistance genes among Gram-negative bacteria especially in *Enterobacteria* and *Pseudomonads* (Hall and Collis, 1998). Antibiotic resistance is a widespread phenomenon and the resistances toward most of the common antibiotics were emerging, thus, continue monitoring of antibiotic resistance pattern among common pathogens in order to combat with this phenomenon is essential. In this present study, in order to identified**,** the extent and prevalence of resistant pattern among the environmental isolates of Gram negative bacteria against different groups of antibiotics were tested by using disk diffusion method.

MATERIALS AND METHODS
Jabalpur (Latitude: 23.2; Longitude: 79.95; Altitude: 391.) is the second biggest city of Madhya Pradesh. The present sampling had been done within the premises of a 500 bedded hospital A of Jabalpur city, 50 - 100 meter apart from the building and at the area B of Jabalpur city which is situated two kilometres from the hospital area as a control, in duplicate and fortnightly in order to cover all the major season.

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The Andersen 2-stage viable (microbial) particle sampler (2-STG) used for sampling which has been developed for monitoring bioaerosols. For this study, air sampling done on Tryptone Glucose Yeast Extract (TGYE) Agar Medium (Hi Media), and Eosin Methylene Blue (EMB) Agar Medium (Hi Media) with the help of modified two stages Andersen Sampler (Andersen, 1958 and 1966, Pathak and Verma, 2009 and 2010). The sampler placed at one meter height from the ground; operated for two minutes at the site in duplicate at morning hours.

Water samples collected from associated sewage system of hospital, by holding the glass stopper, sterile bottle near its base in the hand and plugging it (necked downward below the surface) and transported to the laboratory in an icebox to avoid unpredictable changes in physiochemical as well as bacteriological characteristics.

The dust and top soil of debris sampled in sterile polythene airtight bags. Processing of samples was done by serial dilution technique (10^{-2} to 10^{-4}) to get only a few cells per mL One mL of inoculums from each dilution poured onto sterilized Petri plates of respective media (TGYE & EMB) at 45 °C by using Pour plate technique (Krieg,1981) and incubated at 37 ± 2 °C for 24 to 48 hrs. Identification of isolates was done by using standard methods and manuals (Baron *et al.*, 1994, Brenner and Farmer, 2005, Collee *et al.*, 1999, Jones and Sackin, 1980,). In this, present studies various classes of antibiotics (penicillin, aminoglycosides, cephalosporin, cyclic polypeptides, quinolones, nitrofurons, sulphonamides and analogues) were tested against the species of Enterobacteriaceae family isolated from air and water of hospital associated environment.

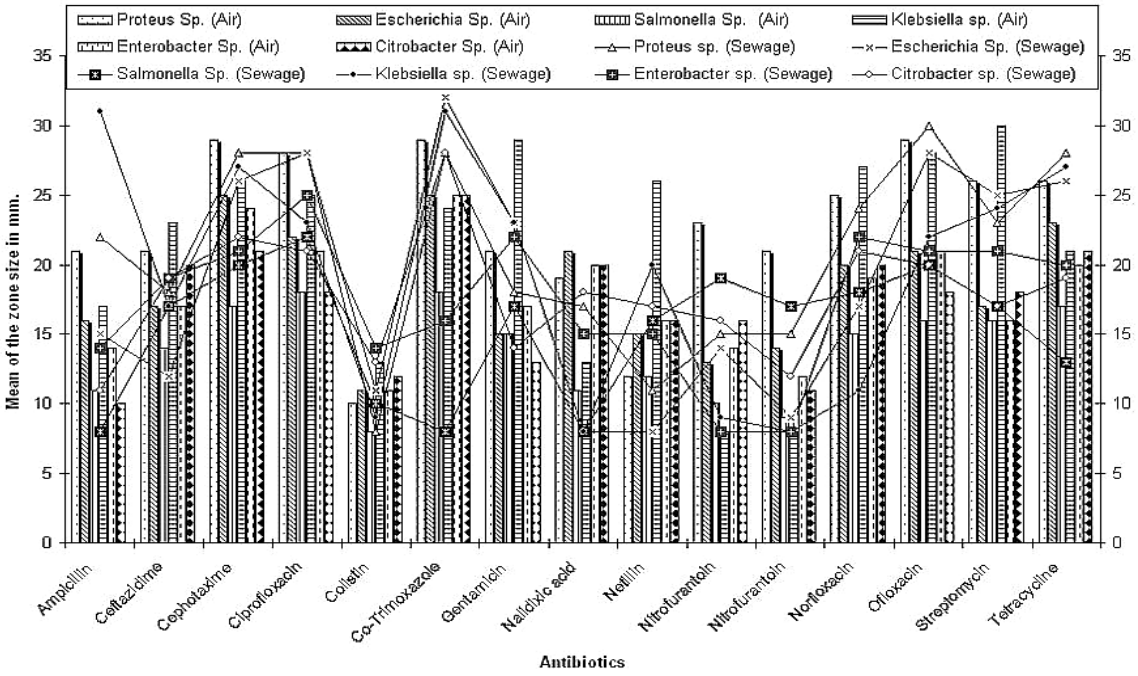

Figure 1: Mean of the zone of inhibition size (in mm.) of some selected species of family Enterobacteriaceae.

Standard method of disk diffusion assay was used to test all environmental isolates. (Collee *et al*., 1999, NCCLS, 2002) The test disks contained ampicillin 10 mcg, ciprofloxacin 10 mcg, colistin 10 mcg, co-trimoxazole 25mcg, Gentamicin 10 mcg, Streptomycin 10 mcg, Tetracycline 30 mcg, Ceftazidime 30 mcg, cephotaxime 30 mcg, nalidixic acid 30 mcg, nitrofurantoin300 mcg, norfloxacin 10 mcg, netillin 30 mcg and ofloxacin 5 mcg (HiMedia, India), stored in desiccators at 4°C. Inoculums were prepared by picking five distinct colonies of approximately 1mm from 24 hours each old culture grown on Tryptone

Glucose Yeast Extract (TGYE) Agar Medium (Hi Media) and incubated at 35±2°C. Colonies are suspended in 5ml of sterile 0.85% Saline. Vortexes the resulting suspension and adjusted the turbidity to yield $1X10^7$ cells/ml (i.e.0.5 McFarland standard). A sterile cotton swab moistened with the inoculum suspension was used to apply to a 90 mm diameter plate containing Mueller-Hinton agar (HiMedia, India). The plates were allowed to dry for 5-15 minutes before disks were placed in the center of the agar. The plates were incubated for 18-24 hours at 37±2°C and the slowly growing isolates were again read after 48 hours incubation. Zone sizes were measured in millimetres with Zone Scale (HiMedia,India), the zone diameter to the nearest whole millimeter the point at which there is prominent reduction in growth were taken into consideration. All the disk diffusion experiments were repeated twice and mean were taken.

Table 1: Antibiogram of some selected species of family Enterobacteriaceae

Antibiotic ↓ Isolates→	Resistant	Intermediate	Sensitive	Proteus sp.(Air)	Proteus sp.(Sewage)	Escherichia sp.(Air)	Escherichia sp.(Sewage)	Salmonella sp.(Air)	Salmonella sp.(Sewage)	Klebsiella sp.(Air)	Klebsiella sp.(Sewage)	Enterobacter sp.(Air)	Enterobacter sp.(Sewage)	Citrobacter sp.(Air)	Citrobacter sp.(Sewage)
Ampicillin	13	14-16	17	S	I	S	S	I	I	**R**	**R**	**R**	I	S	S
Ciprofloxacin	15	16-20	21	S	S	S	S	S	S	S	I	I	S	S	S
Colistin	8	09-10	11	**R**	S	I	I	**R**	S	I	S	**R**	S	S	S
Co-Trimoxazole	10	11-15	16	S	S	S	S	S	S	**R**	S	S	S	S	S
Gentamicin	12	13-14	15	S	S	S	S	S	S	S	I	S	S	S	S
Streptomycin	11	12-14	15	S	S	S	S	S	S	S	S	S	S	S	S
Tetracycline	14	15-18	19	S	S	S	S	S	S	**R**	S	I	S	S	S
Ceftazidime	14	15-17	18	S	I	S	I	**R**	S	I	S	**R**	I	S	S
Cephotaxime	14	15-22	23	S	S	S	S	S	S	I	S	I	S	S	S
Nalidixic acid	13	14-18	19	I	S	S	**R**	**R**	I	**R**	I	**R**	S	**R**	**R**
Nitrofurantoin	14	15-16	17	I	**R**	S	**R**	**R**	**R**	S	**R**	**R**	**R**	**R**	**R**
Norfloxacin	12	13-16	17	S	S	S	**R**	S	S	S	S	I	S	S	S
Netillin	12	13-14	15	**R**	S	I	S	**R**	S	S	S	**R**	S	S	S
Ofloxacin	12	13-15	16	S	S	S	S	S	S	S	S	S	S	S	S

R: Resistance; S: Sensitive; I: Intermediate.

RESULTS AND DISCUSSION

Microbial resistance to significant drugs has become a global public-health problem conciliating the efficiency of antimicrobial chemotherapy. Multi drug resistance (MDR) in bacteria are mediated by chromosomally located resistance determinants and/or mutations in a resident gene. It is observed that a substantial portion of the resistance genes present on the plasmids and transposons of gram-negative

bacilli are integrated into DNA elements called "integrons". These integrons constitute a site specific recombination system capable of integrating and expressing the genes in cassette structures. According to Leverstein-van Hall *et al*. (2003) there is a strong association between MDR and the presence of integrons in Enterobacteriaceae, independent of species or strain origin. The acquisition of resistance genes is not random, and the transfer of integron-carrying elements plays a dominant role in the development of MDR by Enterobacteriaceae. Since, the phenomenon of drug resistance is not restricted to pathogenic bacteria; it also involves all environmental bacteria especially members of the family Enterobacteriaceae due to presence of high mobile elements like integrons.

In this present study species of genera *Proteus*, *Escherichia*, *Salmonella*, *Klebsiella* and *Enterobacter* isolated from air and water of hospital associated environment were tested for drug resistance. In order to identified**,** the extent and prevalence of resistant among members of the family Enterobacteriaceae, different groups of antibiotics were tested against the isolated species (table1).

Extended-spectrum ß-lactamases (ESBL) reported in the species of the genera *Salmonella*, *Klebsiella* and *Enterobacter*. Resistance to quinolones compounds were reported in the species of the genera *Escherichia*, *Klebsiella* and *Enterobacter*. Resistance to amino glycosides found very rare in isolated species, only one strain of *Klebsiella oxytoca* is found resistant to gentamicine but not to streptomycin. Oflaxacin is found most potent antibiotic to Enterobacteriaceae, none of the species were showing resistance toward this drug. Colistin, a cyclic polypeptide found quite ineffective against most of the species tested *in vitro.*

All the species were showing resistance towards nitrofurans, which may be due to its adaptability since this drug is quite effective in case of urinary tract infection caused by Gram negative bacteria. Resistance towards amino glycosides reported in the species of *Proteus* and *Enterobacter*. Resistance to quinolone and quinolone analogues shown by *Escherichia*, *Salmonella*, *Klebsiella*, *Enterobacter* and *Citrobacter*, but none of the species studied were shown resistance towards fluorinated corboxy quinolone compound. *Klebsiella* spp. showing resistance towards sulfonamide and tetracycline, both the property is associated with slightly resistance with cephalosporin and cyclic peptide. Multiple drug resistance reported in *Salmonella* (5 classes), *Klebsiella* (5 classes), *Enterobacter* (6 classes) & Proteus (3 classes).

The ESBLs were reported in the species of *Enterobacter* and *Klebsiella* of air borne in origin from nosocomial environment. Many strains of *Klebsiella* toward ampicillin, chloremphenicol, streptomycin, sulphonamides and tetracycline were reported resistance by Davey and Pittard (1977). According to Delia *et al*. (1976) nalidixic acid and furantiol is highly effective against the species of Enterobacteriaceae, does not supported by the current finding. Cephotaxime is highly effective against *Enterobacter spp.* (Linzenmeier and Rosenthal, 1979) among newer cephalosporin is confirmed by this study. Among quinolone compounds the order of affectivity of drugs against the species of the family Enterobacteriaceae is as follows ofloxacin> ciprofloxacin> norfloxacin> nalidixic acid (Figure1).

Bacterial resistance to antimicrobial agents is a major public health problem in tropical countries (Amyes *et al*., 1992). In Indian subcontinents, the outbreak of multiple drug resistant strain of *Shigella* is already reported (Shears et al., 1995). Numerous factors are responsible for exerting selection pressure and release of multiple drug resistance species in environment i.e. poor sanitary measures (AI-Jebouri and AI-Meshhadani, 1985), indiscriminate use of antibiotics (Romper et al., 2002), Horizontal or Lateral Gene Transfer (Mazodier, and Davies, 1991, Heinemann and Sprague, 1989).

In third world countries the best-suited condition for development of multiple drug resistance is available i.e. poor sanitary measures improper health care, open sewage system etc. This problem required carefully handling and more researches in the area of characterization of Integrons, which have high mobility among the microbial world.

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