PREVALENCE OF ANTIBIOTIC – RESISTANCE BACTERIA IN CHILDREN HOSPITAL

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Abstract:

The discovery of potent antimicrobial agents was one of the greatest contributions to medicine in the 20th century. Unfortunately, the emergence of antimicrobial resistant pathogens now threatens these advances.

The aim of this study was to investigate the Prevalence of Multi Drug Resistant (MDR) Gram-negative and Gram-positive bacteria in Children Hospital, Mansoura University, Egypt. Isolation and identification of resistant Gram-negative and Gram-positive bacteria, Study the mechanism of resistance and isolation of plasmid responsible for resistance that encoded β-lactamases to most multiple resistant bacterial isolates .

The present study was conducted on 200 children. They were subjected to clinical investigations for symptoms such as fever, dysuria, diarrhea, bloody diarrhea, septicemia, and any other urinary tract infections (UTI) in Children Hospital, Mansoura University, Mansoura, Egypt. This study was done over a period of one year from September, 2007 to August, 2008. The source of isolates were urine, stool and blood samples. All isolates were identified and their susceptibility to a number of 35 antimicrobial agents were studied and were examined for the presence of β-lactamases.

The results showed that about 55 % of bacteria isolated (110 isolates) displayed marked resistance to most of antibiotics used. From this study, the isolated Gram-negative bacteria had highly rate of resistant to

ampicillin , ampicillin/sulbactam, cefazolin and cephalothin and the isolated Gram-positive bacteria had highly rate of resistant to azithromycin, penicillin, oxacillin and cefepime. It has been detected that β-lactamase were for penicillin G (90 %) for Gram positive isolates and were for cefotaxime (41.25 %), (43.33 %) for Gram-negative and Gram- positive bacterial isolates respectively. Gram-negative and Gram-positive bacterial isolates that showing highly rate of resistant to antimicrobial agents used were examined for the presence of the plasmid responsible for resistance. All of them give plasmid band at 3 kb.

Conclusion:

We can conclude from this study that Gram-negative bacterial isolates from Children Hospital, Mansoura University, Mansoura, Egypt had highly rate of resistant toward ampicillin ,ampicillin/sulbactam, cefazolin and cephalothin and Gram-positive bacterial isolates had highly rate of resistant toward azithromycin, penicillin, oxacillin and cefepime. This should be considered in antibiotic policy in hospital.

INTRODUCTION

The prevalence of hospital-acquired infections involving multi-resistant bacteria has steadily increased in many countries around the world since 1980. Multidrug resistant bacteria have become endemic in many hospitals, even in institutions that have implemented control strategies designed to limit infections with these bacteria **(Talon, 1999**)

Gram-negative bacilli that belong to the family *Enterobacteriaceae* continue to be the most frequently recovered bacterial isolates from clinical specimens. Thirty named genera are recognized in the family. Although most clinically significant isolates belong to 20 or 25 species that have been well known for many years, new species are being continually discovered **(Warren et al., 2000).**

Gram negative bacilli including *Klebsiella, Citrobacter, Serratia*, *Enterobcter, Proteus species* and *P. aeruginosa* may cause hospital-wide problems because of their ability to acquire resistance to antibiotics. Multiple antibiotic-resistant Gram negative bacilli are more wide spread in the hospital environment as a result of broad-spectrum antibiotic usage and advanced invasive techniques **(Damani, 1997).**

In Gram-positive bacteria, resistance to β-lactam antibiotics may be associated either with a decrease in the affinity of PBP (penicillin binding protein) for the antibiotic or with a change in the amount of PBP produced by the bacterium. In *S.aureus* and *E. faecum*, additional PBPs may be inducible and those have a lower for β-lactam antibiotics **(Opal et al., 2000).**

Bacterial pathogens have become increasingly resistant to avariety of antibiotics. The high usage level of antimicrobial drugs in hospitals provides an atmosphere in which resistant organisms can flourish. For this reason, Gram-positive cocci have become predominant causes of infection **(Hambraeus, 1995).**

The mechanisms of resistance have developed in response to the mechanisms of action of antimicrobial drugs. Antimicrobial agents are generally categorized according to their targeted sites of action.To date, three classes of antibiotic resistance have been described intrinsic resistance, acquired resistance, and genetic resistance **(Hancock, 1998).**

β-lactam antibiotics are antibacterial agents that share the structure feature of a β-lactam ring are known to be very diverse **(Greenwood, 1995).** Resistance to β-lactam antibiotics is due mainly to the production of β-lactamases, enzymes that inactivates these antibiotics by splitting the amide bond of the β-lactam ring.

Numerous β-lactamases exist, encoded either by chromosomal genes or by transferable genes located on plasmids or transposons **(Medeiros, 1984).**

AIM OF THE WORK

The present work was conducted to investigate, 1- Detection of prevalence of Multi Drug Resistant (MDR) Gram-negative and Gram-positive bacteria in Children Hospital, Mansoura University, 2- Isolation and identification of resistant Gram-negative and Gram-positive bacteria, 3- Study the mechanism of resistance and 4- Isolation of plasmid responsible for resistance that encoded β-lactamases to most multiple resistant bacterial isolates .

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MATERIALS AND METHODS

The present study was conducted on 200 children including boys and girls. They were subjected to clinical investigations in Children Hospital, Mansoura University,

Mansoura, Egypt. This study was done over a period of one year. All cases were subjected to clinical examination for symptoms such as fever, dysuria, diarrhea, bloody diarrhea, septicemia, and any other urinary tract infections (UTI) .

Collection of samples:

Urine samples Collection **(Warren,1996; NCCLS, 2001).**

Stool samples Collection **(Cheesbrough, 1993).**

Blood samples Collection **(Warmser et al., 1990).**

Bacteriological examination:

Examination of urine samples:

Physico-chemical examination: **(Hooton and Stamm, 1997).**

Microscopic examination: **(Berry and Schumamm, 1991; Hooton and Stamm, 1997; Wilson and Gaido, 2004).**

Urine samples:

Using a standard bacteriological loop, 0.01ml of uncentrifuged urine sample was inoculated on Cysteine Lysine Electrolyte Deficient (CLED) medium.

By the loop, a single streak was done across the centre of the plate longitudinally. Then, the inoculum was spread evenly at right angles to the primary streak.

After 14-48 hours of incubation at 37°C, the number of bacteria were estimated by counting the number of colonies that appeared on the medium. The number of colonies was multiplied by 100, and this represented the number of colony forming units (CFU) in a milliliter of urine. A count of 1000 or more CFU/ml was considered significant **(Chessbrough, 1993).**

Stool samples:

A loopful of stool was suspended into selenite broth at 37°C for 8h then plated on macConkey agar and bloody diarrheal samples plated on sorbitol macConkey agar media (SMAC), specific for *E.coli* O157:H7 and incubated at 37°C for 24 hours aerobically.

Blood samples:

The inoculated bottle is placed in the incubator at 37°C until growth appear on the agar phase. Subculture was done on blood agar and chocolate agar.

Characterization of bacterial isolates:

Colonal Morphology:

Size, shape, elevation, structure, surface, edge, colour, opacity and consistency.

Microscopic examination:

Preparation of the bacterial smear: **(Collee et al., 1996).**

Gram stain: **(Benson, 1998).**

Microbiological Analysis:

Media used for cultivation, isolation and characterization of bacterial isolates were

CLED Agar, Blood Agar, Selenite broth base (Oxoid kits), MacConkey Agar**,** Sorbitol MacConkey Agar, Nutrient Agar, Chocolate Agar **(Koneman et al., 1997, Koneman et al., 1997, Koneman et al., 1997, Collee et al., 1996 and Koneman et al., 1997).**

dentification of micro organisms:

The bacterial isolates were identified by standard biochemical tests such as Motility Test, Oxidase Test, Citrate utilization test, Indole test, Triple sugar iron (TSI) test, Catalase test, Urease test, Sugar fermentation, Coagulase test**,** Voges proskauer's reaction (V.P.), Methyl red test (M.R.), Gelatin liquefaction **(Washington, 1985, Mac Faddin, 1976; Forbes et al., 1998a, Collee and Marr, 1996, Baron et al., 1994, Koneman et al., 1997, Forbes et al., 1998b, Bowden ,1990 & Wolfgang et al., 1998, Bowden,1990 & Wolfgang et al., 1998, Cheesbrough, 2000, Collee and Marr, 1996, Koneman et al., 1997 and Collee and Marr, 1996).**

Identification of the isolated bacterial strains, after being characterized as previously described were identified to species level following **(Bergey's Manual, 1984, 1986, 1994 & 2005)** and the articles of **(Mahon and Manuselis, 1995; Collee et al., 1996 and Zinsser, 1998).**

The second method for bacterial identification using microscan identification system (DADE BEHRING) system .

Serotyping:

The diagnosis of *E. coli* O157:H7 infection needs to be considered for all patients who present with diarrhea, especially bloody diarrhea or hemolytic uremic syndrome (HUS) **(Griffin et al., 1991), (Belongia et al., 1993 & Pai et al.,1988), (March et al., 1989), (Borczyk et al., 1990) and (Bettelheim et al., 1993 & Corbel et al., 1985).**

Antibiotic sensitivity test: (Bauer et al., 1966).

Tryptone soya broth :**(Koneman et al., 1997).**

Mueller-Hinton medium: **(Koneman et al., 1997).**

Table (1): List of antimicrobial agent used: (Bauer et al., 1966).

The antimicrobial agents listed below including the disc content of antibiotics (Oxoid Hampshire, England).

Antibiotic	Symbol	Disc potency (µg)	Inhibition zone diameter (mm)		
			R	I	S
Amikacin	AK	30	≤ 14	15 - 16	≥ 17
Augmentin	AMC	30	≤ 13	14 - 17	≥ 18
Ampicillin/Sulbactam	SAM	20	≤ 11	12 - 14	≥ 15
Ampicillin	AMP	10	≤ 11	12 - 13	≥ 14
Azithromycin	AZM	15	≤18	19 - 20	≥ 21
Cefazolin	CZ	30	≤ 14	15 - 17	≥ 18
Cefepime	FEP	30	≤ 14	15 - 17	≥ 18
Cefoperazone	CFP	75	≤ 15	16 - 20	≥ 21
Cefotaxime	CTX	30	≤ 14	15 - 22	≥ 23

Cefotetan	CTT	30	≤ 12	13 - 15	≥ 16
Ceftazidime	CAZ	30	≤ 14	15 - 17	≥ 18
Ceftriaxone	CRO	30	≤ 13	14 - 20	≥ 21
Cefuroxime	CXM	30	≤ 14	15 - 17	≥ 18
Cephalothin	KF	30	≤ 14	15 - 17	≥ 18
Gatifloxacin	GAT	5	≤ 14	15 - 17	≥ 18
Gentamycin	GM	30	≤ 12	13 - 14	≥ 15
Chloramphanicol	C	30	≤ 12	13 - 16	≥ 17
Ciprofloxacin	CIP	5	≤ 15	16 - 20	≥ 21
Clindamycin	DA	2	≤ 14	15 - 20	≥ 21
Erythromycin	E	15	≤ 13	14 - 17	≥ 18
Imipenem	IMP	10	≤ 13	14 - 15	≥ 16
Levofloxacin	LVX	5	≤ 27	28 - 40	≥ 41

Linezolid	LZD	30	≤ 20	21 - 22	≥ 23
Meropenem	MEM	10	≤ 16	17 - 18	≥ 19
Moxifloxacin	MXF	4	≤ 15	16 - 18	≥ 19
Ofloxacin	OFX	5	≤ 14	15 - 21	≥ 22
Oxacillin	OXA	1	≤ 10	11 - 12	≥ 13
Penicillin	PEN	10	≤ 26	27 - 46	≥ 47
Piperacillin/Tazobactam	TZP	75/10	≤ 16	17 - 18	≥ 19
Rifampin	RIF	5	≤ 16	17 - 19	≥ 20
Synercid (quinupristin/dalfopristin)	Q/D	15	≤ 15	16 - 18	≥ 19
Tetracycline	TET	30	≤ 25	26 - 28	29 ≥
Ticarcillin/clavulanic acid	TCC	75/10	≤ 18	19 - 22	≥ 23
Tobramycin	TOB	10	≤ 12	13 - 14	≥ 15
Vancomycin	VA	30	≤ 9	10 - 11	≥ 12

The bacterial isolate was designated sensitive (S) intermediate (I) or resistance (R) using the Kirby Bauer interpretative chart **(Bauer et al.,1996).**

Detection of β-lactamase production (Hindler et al., (1994).

All Gram- negative and Gram- positive bacterial isolates that showed resistance to any of the β-lactam antibiotics in routine sensitivity tests were examined for the presence of β-lactamases.

Small-scale preparations of plasmid DNA :

Small-scale preparations of plasmid DNA. Mini preparations of plasmid DNA can be obtained by the alkaline lysis method **Birnboim and Doly (1979) and Ish-Horowicz and Burke (1981).**

Agarose gel electrophoresis of the extracted DNA:

The extracted DNA was fractionated by electrophoresis to resolve the extracted plasmid according to standard protocols **(Davis et al. 1986, EL-Farrash et al., 1997).**

Results

Anumber of 200 bacterial isolates have been isolated from (98/200) 49 % urinary tract infections, (34/200) 17 % diarrhea and (68/200) 34 % sepsis. Samples collected from children patients (hospitalized and outpatients clinics) in Children Hospital, Mansoura University, Mansoura, Egypt.

Fig. (1): Showing the percentage of the source of bacterial isolates.

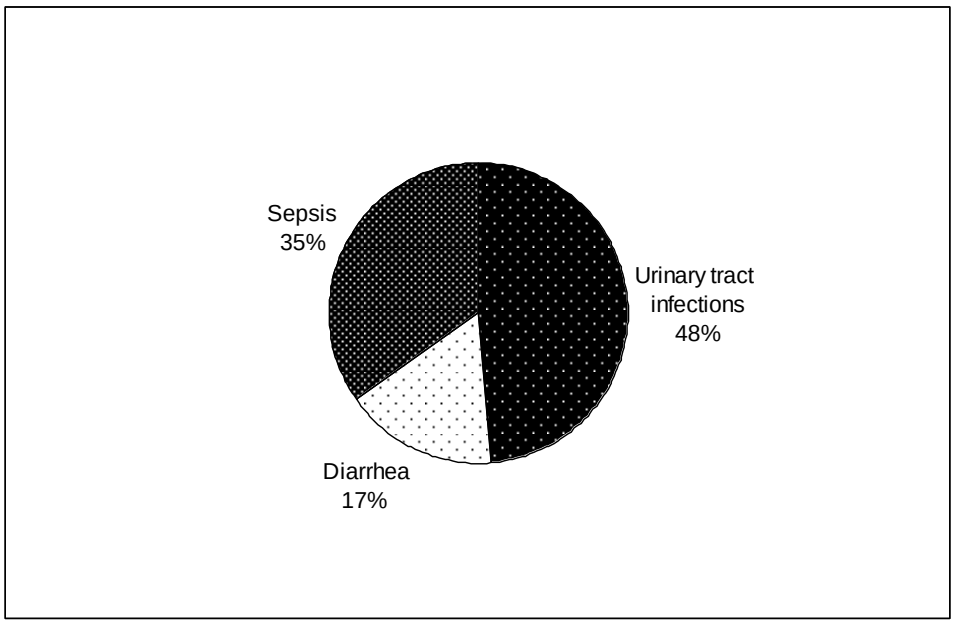


Fig. (2): showing the total Gram-negative bacterial isolates were (132/200) 66% and the total Gram positive bacterial isolates were (68/200) 34%.

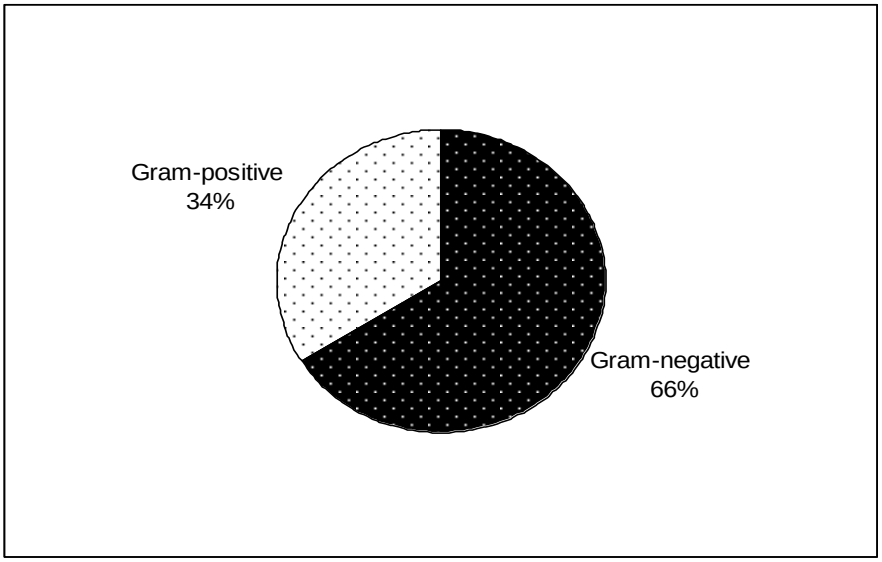


Table (2): Showing the relative frequency of Gram-negative bacterial isolates.

Bacterial species	Number of isolates	%
E.coli non O157:H7	55	41.98
E.coli O157:H7	6	4.58
Klebsiella pneumoniae	27	20.61
Klebsiella oxytoca	2	1.53
Enterobacter cloacae	7	5.34
Enterobacter aerogenes	13	9.92
Citerobacter freundii	8	6.11

Pseudomonas aeruginosa	5	3.81
Pseudomonas fluorescens	2	1.53
Serratia fonticola	4	3.05
Serratia plymuthica	3	2.29
Total	131	100

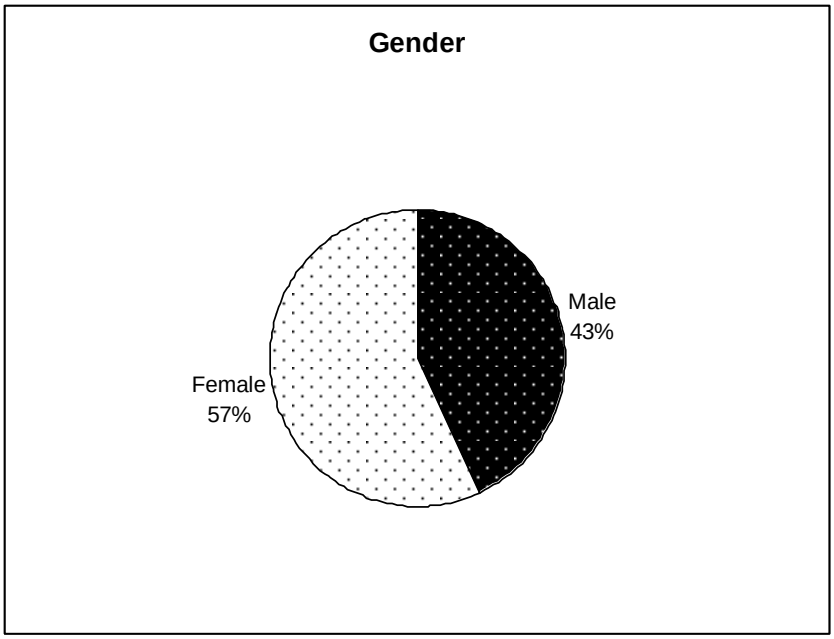
Table (3): Showing the relative frequency of Gram-positive bacterial isolates.

Bacterial species	Number of isolates	%
Staphylococcus aureus	12	17.64
Staph. simulans	7	10.29
Staph. xylosus	9	13.23
Staph. lugdunensis	1	1.47
Staph. auricularis	2	2.94
Staph. cohnii cohnii	6	8.82
Staph. epidermidis	7	10.29
Staph. capitis	7	10.29
Staph. sciuri	2	2.94
Staph. haemolyticus	7	10.29
Micrococcus sp.	2	2.94

Enterococcus faecalis.	6	8.82
Total	68	100

The results showed that the most relative frequency of isolated Gram-negative were 41.98 % for *E.coli* non O157:H7, followed by *Klebsiella pneumoniae* 20.61%, *Enterobacter aerogenes* 9.92%, *Citerobacter freundii* 6.11%, *Enterobacter cloacae* 5.34%, *E.coli* O157:H7 4.58%, *Pseudomonas aeruginosa* 3.81%, *Serratia fonticola* 3.05%, *Serratia plymuthica* 2.29%, *Pseudomonas fluorescens* 1.53%, *Klebsiella oxytoca* 1.53% and the most relative frequency of isolated Gram-positive were 17.64% *Staphylococcus aureus*, followed by 13.23% *Staph. xylosus,* 10.29 % *Staph. simulans*, *Staph. epidermidis*, *Staph. capitis* and *Staph. haemolyticus*, 8.82% *Staph. cohnii cohnii* and *Enterococcus faecalis*, 2.94% *Staph. auricularis*, *Staph. sciuri and Micrococcus sp*. and 1.47% *Staph. lugdunensis*

Fig. (3): Showing the percentage of gender in total Gram-negative and Gram-positive bacterial isolates



The results showed that about 55 % of bacteria isolated (110 isolates) displayed marked resistance to most of antibiotics used as follow:

Table (4): Showing relative frequency of resistant Gram negative bacterial isolates to the various antimicrobial agents used

Table (5): Showing relative frequency of resistant Gram positive bacterial isolates to the various antimicrobial agents used

Bacterial species / Antimicrobial agent	*Staph.aureus*		*Staph.simulans*		*Staph. xylosus*		*Staph. lugdunensis*		*Staph.auricularis*		*Staph. cohnii cohnii*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin / Sulbactam	7	100	4	100	4	100	1	100	2	100	1	100
Ampicillin	7	100	4	100	4	100	1	100	2	100	1	100
Augmentin	7	100	4	100	4	100	1	100	2	100	1	100
Azithromycin	7	100	4	100	4	100	1	100	2	100	1	100
Cefazolin	7	100	4	100	4	100	1	100	2	100	1	100
Cefepime	6	85.71	4	100	4	100	1	100	2	100	1	100
Cefotaxime	7	100	4	100	4	100	1	100	2	100	1	100
Ceftriaxone	7	100	4	100	4	100	1	100	2	100	1	100
Cephalothin	7	100	4	100	4	100	1	100	2	100	1	100
Gatifloxacin	1	14.27	2	50	4	100	0	0	1	50	1	100
Gentamycin	6	85.71	4	100	4	100	1	100	2	100	1	100
Chloramphanicol	4	57.14	2	50	4	100	1	100	1	50	0	0
Ciprofloxacin	2	28.57	3	75	3	75	1	100	2	100	1	100
Clindamycin	6	85.71	2	50	3	75	0	0	0	0	1	100
Erythromycin	6	85.71	4	100	4	100	1	100	2	100	1	100
Imipenem	2	28.57	4	100	4	100	1	100	2	100	1	100
Levofloxacin	2	28.57	3	75	4	100	1	100	1	50	1	100
Linezolid	5	71.43	0	0	3	75	0	0	0	0	1	100
Moxifloxacin	2	28.57	2	50	4	100	0	0	1	50	1	100
Ofloxacin	2	28.57	3	75	4	100	1	100	1	50	1	100

Oxacillin	6	85.71	4	100	4	100	1	100	2	100	1	100
Penicillin	6	85.71	4	100	4	100	1	100	2	100	1	100
Rifampin	6	85.71	1	25	3	75	0	0	0	0	1	100
Synercid	6	85.71	0	0	3		0		0	0	1	100
Tetracycline	7	100	3	75	4	100	1	100	1	50	1	100
Vancomycin	6	85.71	0	0	3	75	0	0	0	0	1	100
Total	7	100	4	100	4	100	1	100	2	100	1	100

Table (6): Showing relative frequency of resistant Gram positive bacterial isolates to the various antimicrobial agents used

Bacterial species / Antimicrobial agent	*Staph. epidermidis*		*Staph. capitis*		*Staph. sciuri*		*Staph. haemolyticus*		*Micrococcus sp.*		*Enterococcus faecalis*	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin / Sulbactam	7	100	4	100	4	100	2	100	1	100	3	100
Ampicillin	7	100	4	100	4	100	1	50	1	100	3	100
Augmentin	7	100	4	100	4	100	2	100	1	100	3	100
Azithromycin	7	100	4	100	4	100	2	100	1	100	3	100
Cefazolin	7	100	4	100	4	100	2	100	1	100	3	100
Cefepime	6	85.71	4	100	4	100	2	100	1	100	3	100
Cefotaxime	7	100	4	100	4	100	2	100	0	0	3	100
Ceftriaxone	7	100	4	100	4	100	2	100	0	0	3	100
Cephalothin	7	100	4	100	4	100	2	100	0	0	3	100
Gatifloxacin	1	14.27	2	50	4	100	1	50	0	0	0	0
Gentamycin	6	85.71	4	100	4	100	2	100	0	0	3	100
Chloramphanicol	4	57.14	2	50	4	100	2	100	1	100	1	33.3
Ciprofloxacin	2	28.57	3	75	3	100	2	100	0	0	3	100
Clindamycin	6	85.71	2	50	3	100	2	100	1	100	3	100
Erythromycin	6	85.71	4	100	4	100	2	100	1	100	2	66.7
Imipenem	2	28.57	4	100	4	100	2	100	0	0	3	100

Levofloxacin	2	28.57	3	75	4	100	0	0	0	0	0	0
Linezolid	5	71.43	0	0	3	100	0	0	0	0	3	100
Moxifloxacin	2	28.57	2	50	4	100	1	50	0	0	1	
Ofloxacin	2	28.57	3	75	4	100	2	100	0	0	1	33.3
Oxacillin	6	85.71	4	100	4	100	2	100	0	0	0	0
Penicillin	6	85.71	4	100	4	100	2	100	1	100	3	100
Rifampin	6	85.71	1	25	3	100	0	0	1	100	2	
Synercid	6	85.71	0	0	3	100	0	0	0	0	3	100
Tetracycline	7	100	3	75	4	100	2	100	0	0	3	100
Vancomycin	6	85.71	0	0	3	100	0	0	0	0	3	100
Total	7	100	4	100	4	100	2	100	1	100	3	100

Table (7): Relative frequency of number of antibiotics that Gram negative bacterial isolates resistant to it.

Bacterial species resistant to antibiotics	Number of antibiotics n=20	%
(1) E. coli n= (21)	9	45
(2) E. coli O157:H7 n= (6)	9	45
(3) Klebsiella pneumoniae n= (13)	9	45
(4) Klebsiella oxytoca n= (1)	13	65
(5) Enterobacter cloacae n= (6)	13	65
(6) Enterobacter aerogenes n= (12)	15	75
(7) Citrobacter freundii n= (7)	15	75
(8)Pseudomonas aeruginosa n= (5)	13	65
(9)Pseudomonas fluorescens n= (2)	8	40
(10) Serratia fonticola n= (4)	15	75
(11) Serratia plymuthica n= (3)	9	45
Total	20	100

Table (8): Relative frequency of number of antibiotics that Gram positive bacterial isolates resistant to it.

Bacterial species resistant to antibiotics	Number of antibiotics n=26	%
(1) Staphylococcus aureus n=(7)	20	76.9
(2) Staph.simulans n= (4)	18	69.23
(3) Staph.xylosus n= (4)	26	100
(4) Staph lugdunensis n= (1)	19	73.17
(5) Staph.auricularis n= (2)	14	53.85
(6) Staph.cohnii cohnii n= (1)	25	96.15
(7) Staph.capitis n= (2)	15	57.69
(8) Staph.epidermidi n= (2)	17	65.38
(9) Staph.sciuri n= (1)	25	96.15
(10) Staph.haemolyticus n= (2)	19	73.17

(11) Micrococcus sp. n= (1)	9	34.62
(12) Enterococcus faecalis n= (3)	19	73.17
Total	26	100

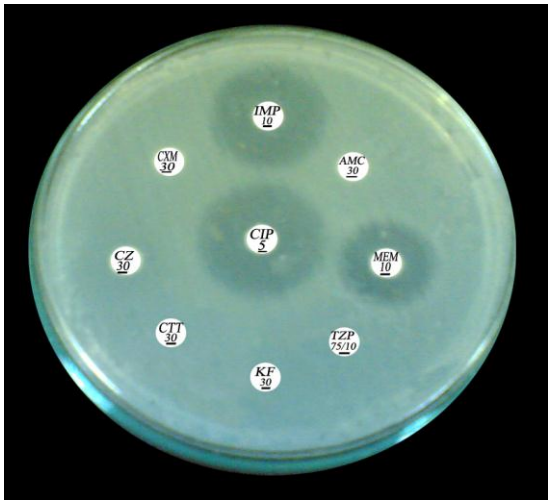


Photo 1: Antibiotic sensitivity test for isolated *Enterobacter aerogenes* by disc diffusion method on Muller-Hinton medium **(Koneman et al., 1997).**

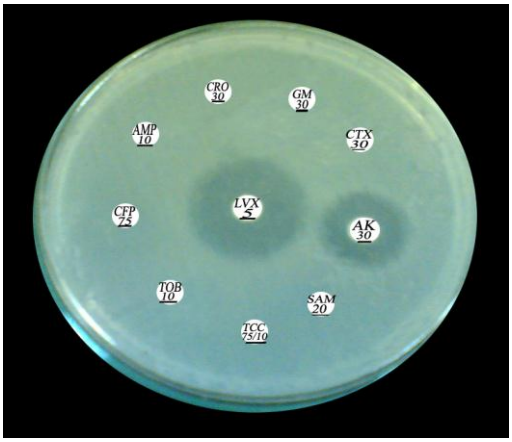


Photo 2: Antibiotic sensitivity test for isolated *Enterobacter aerogenes* by disc diffusion method on Muller-Hinton medium **(Koneman et al., 1997).**

Fig. (4): Showing β-lactamase frequency with cefotaxime in Gram-negative bacterial isolates.

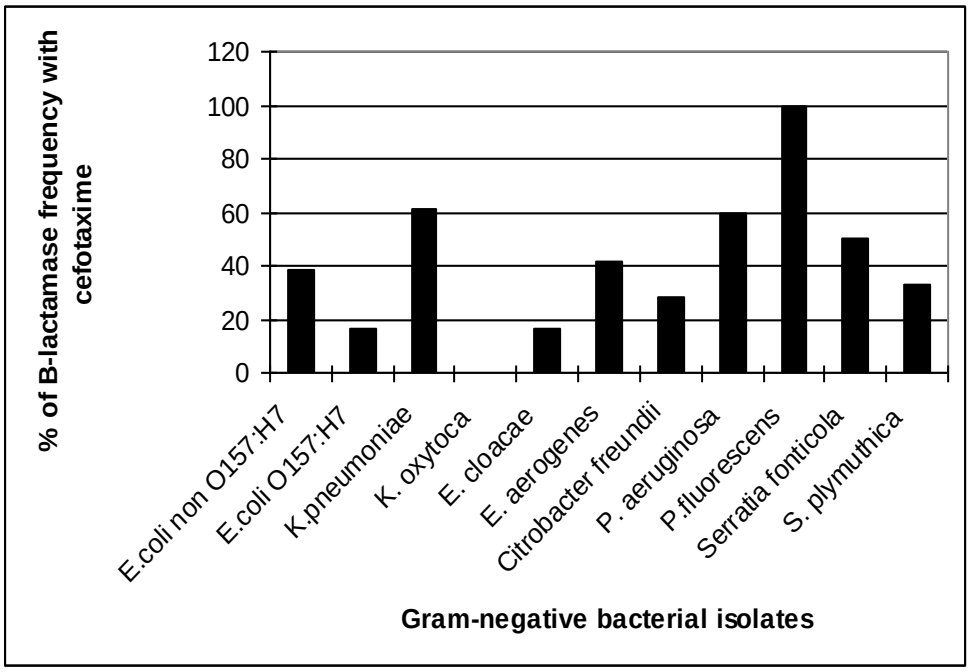


Fig. (5): Showing β-lactamase frequency with cefotaxime in Gram-negative bacterial isolates.

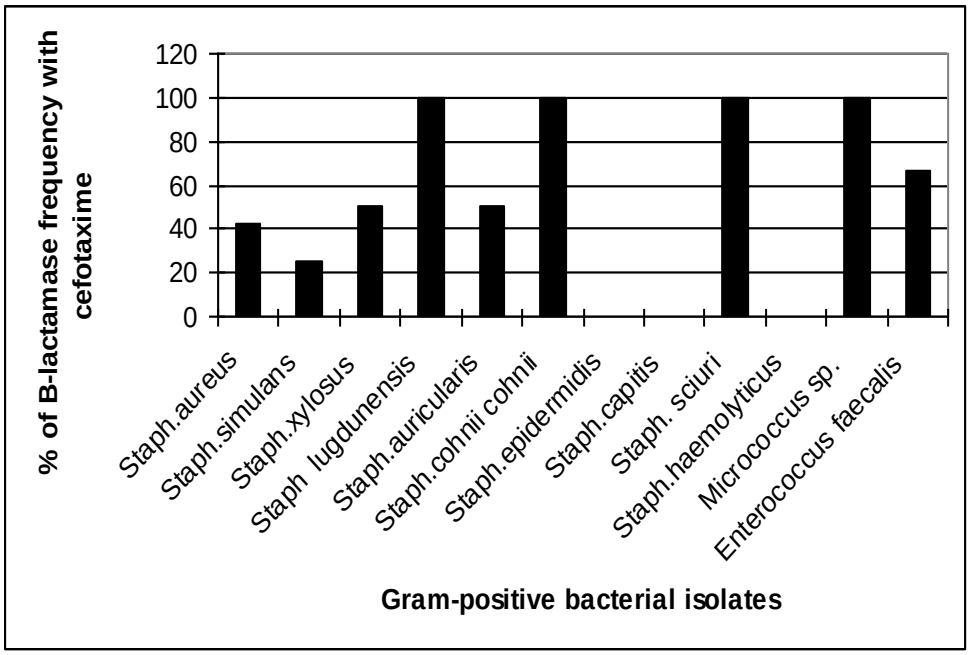
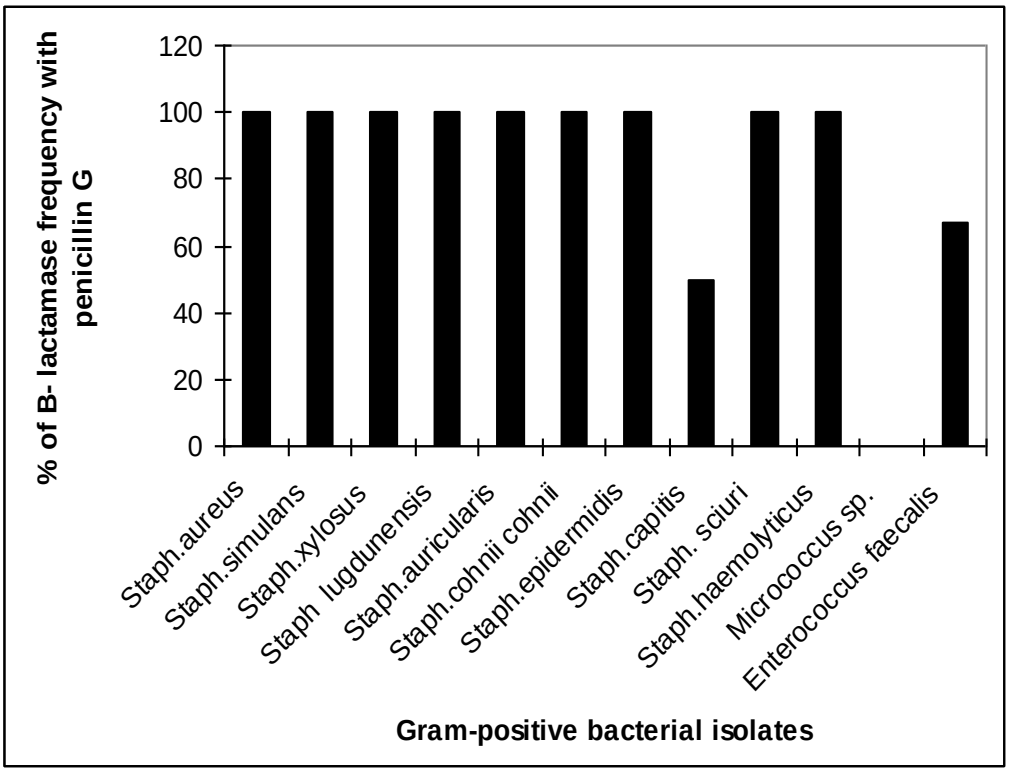
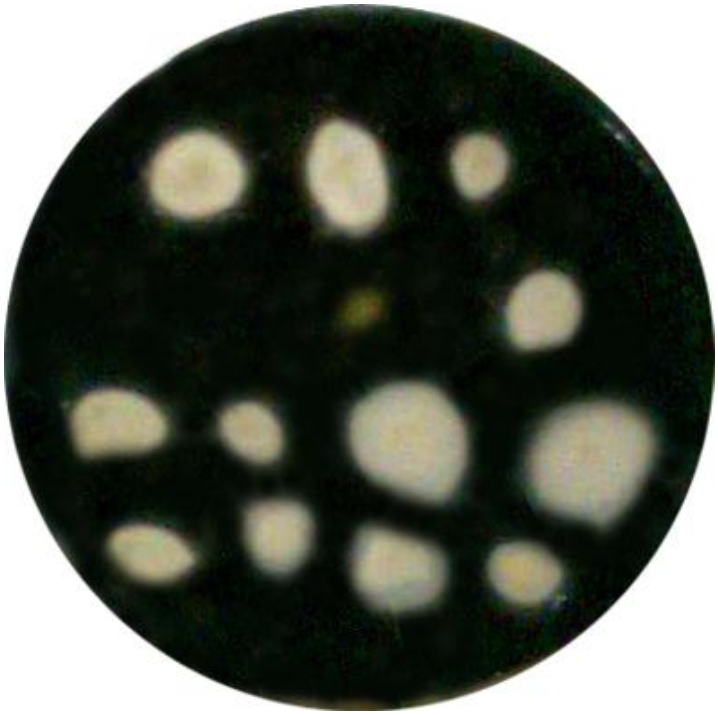


Fig. (6): Showing β-lactamase frequency with penicillin G in Gram-positive bacterial isolates.



From this test (β-lactamase detection), when the test give positive results then must not give the patients any of β-lactam antibiotics.

Photo 3: Showing β-lactamase test with penicillin G in Gram-positive bacterial isolates (Colorless zone give positive result), the isolates were cultured from left to right as follow : 16 (*Staph. simulans*), 17 (*Staph. auricularis*), 18 (*Staph. aureus*), 19 (*Micrococcus sp*.), 20 (*Enterococcus faecalis*), 21 (*Enterococcus faecalis*), 22 (*Staph. simulans*), 23 (*Staph. xylosus*), 24 (*Staph. aureus*), 25 (*Staph. aureus*), 26 (*Staph. epidermidis*), 27 (*Staph. simulans*), 28 (*Staph. auricularis*) , 29 (*Enterococcus faecalis)*, 30 (*Staph. aureus*).



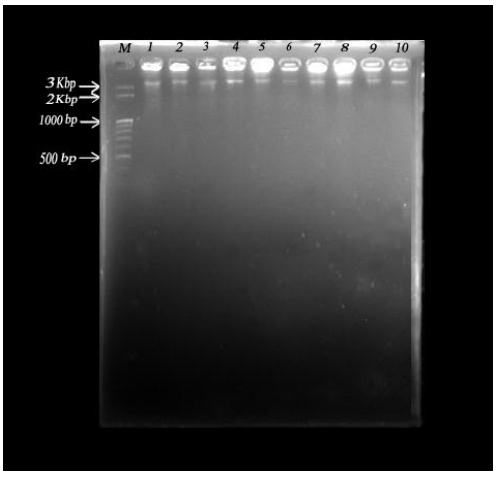
Showing positive β-lactamase in isolates no. 16, 17, 18, 21 (weak result), 22, 23, 24, 25,26, 27, 28, 29, 30.

Photo 4: Showing β-lactamase test with Cefotaxime in Gram-negative bacterial isolates (Colorless zone give positive result), the isolates were cultured from left to right as follow: 25 (*E. aerogenes*), 26 (*E.aerogenes*), 27 (*E. aerogenes*), 28 (*E. aerogenes*), 29 (C. freundii), 30 (*C. freundii*), 31 (*P. aeruginosa*), 32 (*P. fluorescens*), 33 (*S. fonticola*), 34 (*S. fonticola*), 35 (*S. fonticola*), 36 (*S. pylmuthica*), 37 (*E. coli*), 38, (*P.aeruginosa*), 39 (*K. pneumoniae*), 40 (*C. freundii*).

Showing positive β-lactamase in isolates no. 25, 26, 27, 28, 29, 32, 33, 34,36,38,39,40

Photo 5: Plasmid minipreb. from the most 10 Gram-negative and Gram-positive bacterial isolates showing resistant to antimicrobial agents used.This bacterial isolates were *Enterobacter aerogenes* (1), *Enterobacter cloacae* (2), *Pseudomonas aeruginosa* (3), *Citrobacter freundii* (4), *Serratia fonticola* (5), *Staphylococcus aureus* (6), *Staph*. *xylosus* (7), *Staph.sciuri* (8), *Staph.cohnii cohnii* (9), *Enterococcus faecalis* (10).

M: DNA marker



All of bacterial isolates give plasmid band at 3 kb.

Discussion

Antimicrobial resistance is a predictable outcome of antimicrobial use. The rapidity with which resistance emerges and its extent are proportional to the intensity of antimicrobial use **(WHO, 2001**

In 2002, a new programme of community action in the field of public health (2003-2008) was adopted **(OJ L, 2003-2008).** This programme provides an annual public health work plan and a funding mechanism for projects addressing priorities such as antimicrobial resistance. Over recent years emphasis has been given to surveillance initiatives, whereas the focus of future public health work plans may need to be broadened. Activities that develop principles and guidelines for good practice on the prudent use of antimicrobial agents are needed as well as educational activities and intervention programmes to combat antimicrobial resistance.

From our results, the source of most bacterial isolates were from urinary tract infections (48 %) followed by sepsis (35%) followed by diarrhea (17%).

The results showed that the total isolates were 200 bacterial isolates with 57% female and 43% male. The Gram-negative bacterial isolates were (66%) more than Gram-positive bacterial isolates (34%). Similarly to our results, In a study in U.S. hospitals during the year 2003 reported that Gram-positive cocci contributed (32.5%)

isolates and Gram-negative bacilli accounted for (67.5%) isolates. The frequency of micro-organisms identified in nosocomial infections at Unicamp University Hospital from 1987 to 1994 was analysed. The most common were Gram-negative rods 56.5% isolated from urinary tract infections and *Staph. aureus* 20.9%, which was found in blood stream and arterial-venous inections.

Contradictory to our finding, During the last decade, there have been reports about increase prevalence of Gram-positive bacteria from nosocomial infections, especially from bloodstream infections **(Weber, 1992).**

Antibiotic use provides selective pressure favoring resistant bacterial strains; inappropriate use increases the risk for selection and dissemination of antibiotic-resistant bacteria, which are placed at a competitive advantage. Therefore, one would expect that drugs more commonly affected by bacterial resistance in developing countries are generally inexpensive and popular broad-spectrum agents **(Hoge et al., 1998 and Calva et al., 1996).** However, the relationship between antibiotic use and the emergence and spread of resistance is complex. Antibiotic use in clinical practice alone cannot explain the high frequency of resistant organisms in developing countries **(Unin, 1990).**

From our results, the most relative frequency of isolated Gram-negative bacteria were 41.98 % for *E.coli* non O157:H7, followed by *Klebsiella pneumoniae* 20.61%, *Enterobacter aerogenes* 9.92%, *Citerobacter freundii* 6.11%, *Enterobacter cloacae* 5.34%, *E.coli* O157:H7 4.58%, *Pseudomonas aeruginosa* 3.81%, *Serratia*

fonticola 3.05%, *Serratia plymuthica* 2.29%, *Pseudomonas fluorescens* 1.53%, *Klebsiella oxytoca* 1.53% and the most relative frequency of isolated Gram-positive were 17.64% for *Staphylococcus aureus*, followed by 13.23% *Staph. xylosus,* 10.29 % *Staph. simulans*, *Staph. epidermidis*, *Staph. capitis* and *Staph. haemolyticus*, 8.82% *Staph. cohnii cohnii* and *Enterococcus faecalis*, 2.94% *Staph. auricularis*, *Staph. sciuri and Micrococcus sp*. and 1.47% *Staph. lugdunensis*. Similarly to our results, In a study in hospitals in Benin City, Nigeria, from June 2001 to September 2005, the screened isolates include *E coli* (26.8%), *Pseudomonas aeruginosa* (18.7%), *Klebsiella pneumoniae* (15.4%), *Salmonella typhi* (11.4%), *Shigella dysenteriae* (10.5%), *Proteus mirabilis* (9.9%) and *Serratia marcescens* (7.3%) from urinary tract infections **(Yah et al., 2006).** However, in another study in three health institutions in Nigeria on skin and soft-tissue infections. The screened isolates include *Staphylococcus aureus*, *Staph. sciuri, Staph. haemolyticus* and *Staph. epidermidis* accounts the majority of coagulase negative staphylococcus infections **(Charalambous et al., 2003).** Contradictory to our results, *Staph. xylosus* were accounted the majority of isolated coagulase negative staphylococcus infections.

(Johnson et al., 1998) showed that, multi drug resistant (MDR) Gram-negative *Klebsiella pneumoniae* and *K.oxytoca* that resistant to cephalosporins, aminoglycosides, fluoroquinolones, and other MDR enterobacteriaceae including *Escherichia coli*, *Serratia* spp., *Proteus* spp. and *Enterobacter* spp. that resistant to cephalosporins, aminoglycosides, fluoroquinolones, and carbapenems resistant *Pseudomonas spp.* are likely to become a major clinical problem remains unclear.

Similarly, we were found that, the isolated Gram-negative bacteria had highly rate of resistance to ampicillin , ampicillin/sulbactam, cefazolin and cephalothin and the isolated Gram-positive bacteria had highly rate of resistance to azithromycin, penicillin, oxacillin and cefepime.

The main cause of resistance to cephalosporins are the production of β-lactamases that inactivates these antibiotics by splitting the amide bond of the β-lactam ring. Resistance to β-lactam antimicrobial agents, especially extended-spectrum cephalosporins and other antimicrobial agents among clinical isolates of Gram-negative bacteria, is on the rise worldwide **(Pfaller, 1999).**

In 2001, the World Health Organization (WHO) launched the first global strategy to counter this phenomenon, a key component of which is the development of surveillance programs to monitor in antimicrobial drug resistance and use **(Simonsen, 2004).**

In our results, the most relative frequency of resistance to antibiotics used were 75% for *Enterobacter aerogenes*, *Citrobacter freundii* and *Serratia fonticola*, 65% for *Pseudomonas aeruginosa, Klebsiella oxytoca* and *Enterobacter cloacae,* 100% for *Staph. xylosus*, 96.15% for *Staph. cohnii cohnii* and *Staph. sciuri* and 76.9% for *Staph. aureus*. Similarly to our results, studies in Taiwan have demonstrated

a high prevalence of Gram-negative antimicrobial resistant bacteria and a trend of increasing resistance under continued antibiotic selective pressure **(Hsueh et al., 2002).** These antimicrobial pathogens include extended-spectrum cephalosporin- or fluoroquinolone- resistant *E.coli* non O157:H7, *Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens* and *Citrobacter freundii*, and carbapenem- or ciprofloxacin- resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* **(Jan, 1998).**

Contradictory to our results, in both United States and Canada, *Staph. aureus* and *Escherichia coli* were the most common resistant, followed by coagulase negative staphylococci and enterococci. *Klebsiella spp., Enteobacter spp., Pseudomonas aeruginosa, Streptococcus pneumoniae* and β-hemolytic streptococci were also the most frequently reported species in both the United States and Canada.

The difference between our results and results in the United States and Canada can be attributed to misuse of antibiotics in both community and hospital-acquired infections with out medical prescription.

Failure to stop unnecessary antimicrobial treatment contributes to overuse and resistance, so the uses of antibiotics should be wisely.

Recent Centers for Disease Control and Prevention (CDC) data show that in 2002, nearly 33 percent of tested samples of *Pseudomonas aeruginosa* from ICUs were resistant to fluoroquinolones. Similarly, in our results *Pseudomonas aeruginosa* isolates were resistant to fluoroquinolones as ciprofloxacin (42.86%).

The more important emerging resistance problems are oxacillin resistance in staphylococci, penicillin resistance in streptococci, vancomycin resistance in enterococci (and eventually staphylococci), resistance to extended-spectrum cephalosporins and fluoroquinolones in the enterobacteriaceae, and carbapenem resistance in *Pseudomonas aeruginosa* **(Archiabald, 1997 and Pfaller, 1997).** Similarly, in our results all *Staphylococcus spp*. were oxacillin resistance and penicillin resistance, all *Micrococcus sp.* were penicillin resistance and all *Enterococcus sp*. were penicillin resistance and vancomycin resistance and among enterobacteriaceae, *Pseudomonas aeruginosa* show little rate of resistance to carbapenem and highly rate of resistance to cephalosporins and other enterobacteriaceae show highly rate of resistance to cephalosporins and some species show resistance to fluoroquinolones.

From our results, *Enterobacter cloacae* and *Enterobacter aerogenes* isolates showed highly rate of resistance to ceftazidime than similar data from Europe and the USA **(Pfaller, 2000**

Mathai et al., 2001 reported that among pulmonary isolates of *Enterobacter* spp. from the USA, only 79.6% were susceptible to ceftazidime and 100% to imipenem. Condactory to our results, all isolates of *Enterobacter cloacae* and *Enterobacter aerogenes* were 100% ceftazidime resistant. However, We found that *Enterobacter cloacae* isolates were 100% susceptible to imipenem and meropenem and *Enterobacter aerogenes* isolates were 92.6 % susceptible to imipenem and 33.3% to meropenem.

Screening of more than 200 isolates of CoNS from cultures of blood from neonates in the VU University Medical Center NICU showed that *Staph. capitis* strain had been endemic in the unit since 1998 and that it was the causative agent of about one-third of all cases of bacteremia caused by CoNS in the VU University Medical Center NICU. This study was initiated after the detection of a case of ongoing sepsis caused by CoNS in a premature neonate, as determined by several positive blood cultures. Treatment with vancomycin was not effective. The sepsis was caused by a strain of *Staph. capitis* which was heteroresistant to vancomycin. Although the precise genetic mechanism for vancomycin resistance in staphylococci awaits elucidation, it is considered that thickening of the cell wall peptidoglycan layer is responsible. Vancomycin is captured in the thickened cell wall and is prevented from reaching its targets on the cell membrane

(Hanaki and Hiramatsu, 1998 and Sieradzki and Tomasz, 1997). Contradictory to our results *Staph. capitis* was the causative agent of about 10.29% of all cases of bacteremia caused by CoNS and show

highly rate of resistance to β-lactam antibotics aminoglycosides, and macrolides and show sensitivity toward vancomycin, synercid and linzolid

Wu et al., 2001 reported that, the isolation of multiresistant *Staph. sciuri* from clinical sources is important not only because of its serious impact on the course of infection, but because this species is a potential source of genes encoding resistance to antibiotics for other staphylococci pathogenic for man. Similarly to our results, *Staph. sciuri* was the causative agent of about 3% of all cases of bacteremia caused by CoNS and were 100% resistant to all antibiotics used.

Tabe et al., 1998 reported that, *Staph. haemolyticus* has been associated with septicaemia in neonates and various infections in individuals with compromised host defences and implanted foreign bodies. The emergence of strains with decreased susceptibility to vancomycin has been reported in health institutions. It is highly prevalent in the hospital environment, with a tendency to develop resistance to multiple antibiotics. Similarly to our results, *Staph. haemolyticus* was the causative agent of about 10.29% of all cases of septicaemia caused by CoNS and had highly rate of resistance to penicillins, cephalosporins, aminoglycosides and fluoroquinolones and were 100% susceptible to levofloxacin, linezolid, rifampin, synericid and vancomycin.

Prevalence of vancomycin-resistant enterococci (VRE) in U.S. hospitals is estimated to be roughly 12% on average across all hospital patients **(McDonald 2006)** and according to the CDC is more than 28% in intensive care units**(CDC 2004).** In Europe, only Portugal had a higher prevalence of VRE than did the United States. Data on VRE prevalence outside Europe are less reliable but show lower rates than the United States, with the exception of South Korea. Reliable studies from Japan have found isolated outbreaks but no evidence of VRE transmission **(Matsumoto et al., 2004).** In contrast to reports from many parts of the world, <5% of enterococcal isolates were vancomycin resistant **(Low et al., 2001).** Approximately 18% of U.S. isolates of *Enterococcus spp.* were resistant to vancomycin versus 0% of Canadian isolates. Contradictory to our results, *Enterococcus feacalis* isolates were (100%) vancomycin resistant. However, *Enterococcus feacalis* isolates were 100% susceptible to levofloxacin, gatifloxacin and oxacillin.

We found from our results that β-lactamase were (90 %) for penicillin G for Gram positive bacterial isolates and for cefotaxime were (41.25%) for Gram-negative bacterial isolates and (43.33%) for Gram-positive bacterial isolates. Similarly to our results, **(Bannerman et al., 2003)** reported that Penicillin G-resistant *S. aureus* strains from clinical infections always produce penicillinase. They now constitute about 90% of *S. aureus* isolates in communities in the United States. They are often susceptible to β-lactamase-resistant penicillins, cephalosporins, or vancomycin, contradictory to our results,

(Felmingham et al., 2000) who reported that ampicillin resistance, which is usually due to β-lactamase production was observed in 15.1% of UK isolates in 1995, whilst β-lactamase production rates of 20–30% have been found in Spain, Hong Kong, and North America

We found from our results that, the most Gram-negative bacterial isolates that produce β-lactamase enzymes with cefotaxime were *Pseudomonas fluorescens* 100 %, and the most Gram-positive bacterial isolates that produce β-lactamase enzymes with cefotaxime were *Staph lugdunensis*, *Staph.cohnii cohnii*, *Staph.sciuri* and *Micrococcus sp.* by percentage 100% and the most Gram-positive bacterial isolates that produce β-lactamase enzymes with penicillin G were *Staphylococcus aureus*, *Staph. simulans*, *Staph. xylosus*, *Staph. lugdunensis*, *Staph. auricularis*, *Staph. cohnii cohnii*, *Staph. epidermidis*, *Staph. sciuri* and *Staph. haemolyticus* by percentage 100%.

From this test (β-lactamase detection), when the test give positive results then must not give the patients any of β-lactam antibiotics.

Numerous β-lactamases exist, encoded either by chromosomal genes or by transferable genes located on plasmids or transposons **(Medeiros, 1984).**

From this study, we detected that Gram-negative and Gram-positive bacterial isolates that showing highly rate of resistanc to antimicrobial agents

used were examined for the presence of the plasmid responsible for resistance that encoded β-lactamases. All of them give plasmid band at 3 kb.

Gene amplification or mutations at either the promoter and/or the attenuator of the structural β-lactamase gene result in AmpC hyperproduction **(Caroff and Reynaud, 1999).** This causes increased resistance to penicillins, cephalosporins, and β-lactam β-lactamase inhibitor combinations. β-Lactams penetrate into Gram-negative bacteria throughout nonspecific porins **(Nikaido, 1989).** Simirarly to our results, all *staphylococcus spp.* isolates were resistant to ampicillin/sulbactam 100% except *staph. haemolyticus* isolates were 50 %, *Micrococcus sp.* isolates were 100% and *Enterococcus feacalis* isolates were 100%. However, we showed some sensitivity in Gram-negative bacterial isolates such as *Serratia fonticola* and *Klebsiella oxytoca* isolates were sensitive 100% to ampicillin/ sulbactam.

There are other causes of resistance rather than β-lactamases such as resistance to a wide variety of antimicrobial agents including tetracyclines, macrolides, lincosmides and other aminoglycosides, may result from alteration of ribosomal binding sites. Failure of the antibiotic to inhibit protein synthesis and cell growth. Resistance occurs as a result of at least eight classes of methylase enzymes that dimethylate adenine residues on the 23S ribosomal RNA of the 50-S subunit of prokaryotic ribosome **(Opal et al., 2000).**

The target of aminoglycoside activity in the bacterial cell is the 30S ribosomal subunit. When the drug binds to the ribosome, the structure is unable to translate mRNA for protein production, leading to cell death. Resistance typically results from drug inactivation by plasmid- or chromosome-encoded enzymes harbored by resistant strains, although enzyme-independent resistance resultant from defects in uptake and accumulation (dubbed impermeability resistance) is also commonplace, particularly in isolates from CF patients **(Miller et al., 1995)** and intensive care units (ICUs) **(Goossens, 2003).**

Carbapenem resistance is usually due to reduced bacterial uptake (due to the loss of the OprD2 porin), or increased efflux, and is often low-level **(Henwood et al., 2000).**

An efflux system, involving three proteins (Mex A, Mex B, and Opr M) is critical for the intrinsic resistance of *P. aeruginosa*. Mutation in any of the genes encoding these proteins led to a fourfold to tenfold increase in susceptibility to quinolones, β-lactams (except imipenem), tetracycline, and chloramphanicol **(Poole et al.,1993).**

Resistance may be result from a change in membrane permeability that makes the drug unable to penetrate through the membrane into the cell. This may be due to a change in structural protein, a decrease in pore size or an alteration in the transport system.

Alternatively the rate of efflux of the drug from the cell may be increased making the drug unable to attain a sufficiently high concentration inside the cell to cause inhibition. This is the basic of resistance to tetracyclines **(Delgadillo et al., 1993).**

The potential importance of vaccines in controlling life threatening infection must not be underestimated and research should be encouraged since vaccines may become increasingly important in preventing infections due to multi-resistant pathogens. Vaccines do not suffer the problem of resistance because a vaccines enhances the body's natural defenses, while an antibiotic operates separately from the body's normal defenses. Nevertheless, new strains may evolve that escape immunity induced by vaccines.

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