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DISTRIBUTION OF MULTIDRUG-RESISTANT GRAM-NEGATIVE BACTERIAL INFECTION IN HOSPITALIZED INFANTS

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ABSTRACT

Sixty-five Gram-negative bacterial isolates were collected from different samples (blood, urine, stool, cerebrospinal fluid (CSF) and wound swabs) from Mansoura University Children's Hospital, and identified as *Klebsiella pneumoniae* (*n*= 10), *Escherichia coli* (*n*= 8), *Enterobacter aerogenes* (*n*= 7), *Enterobacter cloacae* (*n*= 6), *Serratia fonticola* (*n*= 6), *Citrobacter freundii complex* (*n*= 5), *Kluyvera ascorbata* (*n*= 4), *Acinetobacter baumannii/haemolyticus* (*n*= 3), *Pseudomonas aeruginosa* (*n*= 2), *Pseudomonas fluorescens/putida* (*n*= 2), *Vibrio alginolyticus* (*n*= 2), *Vibrio damsela* (*n*= 1), *Cedecea lapagei* (*n*= 1), *Cedecea species 3* (*n*= 1), *Proteus mirabilis* (*n*= 1), *Providencia stuartii* (*n*= 1), *Serratia marcescens* (*n*= 1), *Serratia plymuthica* (*n*= 1), *Serratia liquefaciens* (*n*= 1), *Stenotrophomonas maltophilia* (*n*= 1) and *Acinetobacter lwoffii* (*n*= 1). Thirty-one antimicrobial agents were tested against the isolated bacteria. Gatifloxacin, imipenem and meropenem were found to be the most effective agents against the isolated bacteria, while ampicillin, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, gentamicin and tobramycin were found to be of low effect against the isolated bacteria.

INTRODUCTION

Gram-negative bacilli are the leading cause of serious infections in both the community and hospital settings (Jarvis *et al.,* 1991). Gram-negative bacteria of the Enterobacteriaceae family are important causes of urinary tract infections (UTIs), bloodstream infections, and various intra-abdominal infections (Paterson, 2006; Tan, 2008). Enterobacteriaceae are a large, heterogeneous family consists of 41 genera of Gram-negative, facultatively anaerobic bacteria (Holcombe and Schauer, 2007). Enterobacteriaceae often are labeled as 'enteric bacteria' because of their predilection for intestinal colonization. According to the latest classification by Ewing (1986), a number of genera within the family are major human intestinal pathogens (e.g., *Shigella, Salmonella*), and several are normal colonizers of the human gastrointestinal tract (e.g., *Escherichia, Klebsiella*) (Eisenstein and Zaleznik, 2000). *E. coli* typically colonizes the gastrointestinal tract of human infants within a few hours after birth (Kaper *et al.,* 2004). Non-fermenting Gram-negative bacteria (non-fermenters) are widespread in the environment and are an increasing cause of serious infections in hospital practice. Non-fermenters comprise numerous species belonging to many genera. The three species most often causing significant problems in hospital practice, namely *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* (Enoch *et al.,* 2007; McGowan, 2006).

The urinary tract is one of the most common sites of Gram-negative bacterial infection in humans. Infants and children are also susceptible to UTIs. Pediatric UTIs might predispose patients to adult disease (Nielubowicz and Mobley, 2010). Whereas Gram-negative bacteria are relatively unusual blood culture contaminants, Gram-negative bacteria may enter the bloodstream intermittently or be present in very low numbers (Munford, 2006). In several case series of patients with Gram-negative bacteremia, Enterobacteriaceae and Pseudomonadaceae were isolated from blood cultures in more than 90% of the episodes (Sands *et al.,* 1997). In general, the spectrum of Gram-negative bacteria in positive blood cultures of patients with severe sepsis has been similar to that found in less-ill patients (Brun-Buisson *et al.,* 1996).

Antimicrobial susceptibility testing of bacterial pathogens is one of the primary functions of a diagnostic microbiology laboratory (Sundsfjord *et al.,* 2004). The global emergence and spread of antimicrobial resistance poses a major risk for human health due to the impact on morbidity, mortality, and health care costs (McGowan, 2001). Antimicrobial surveillance programmes provide a wealth of valuable information on the pattern and development of bacterial resistance in different geographical regions. Surveillance programme data evaluation allows changes in antimicrobial prescribing practices, which ultimately minimises the development and spread of antimicrobial resistance genes (Jones and Masterton, 2001). The Study for Monitoring Antimicrobial Resistance Trends (SMART) began in 2002 and exclusively examines the trends in resistance among aerobic and facultatively anaerobic Gram-negative bacilli isolated from intra-abdominal infections (Hoban *et al.,* 2010). The rates of antimicrobial drug resistance and particularly of multiple drug resistance are increasing among Enterobacteriaceae, thus limiting the armamentarium of potentially active antimicrobial agents (Falagas and Bliziotis, 2007). β-Lactams– penicillins, cephalosporins, carbapenems and monobactams– represent 60% of all antimicrobial use by weight. They are preferred because of their efficacy and safety and because their activity can be extended or restored by chemical manipulation (Livermore and Woodford, 2006).

Resistance to the expanded-spectrum cephalosporins can occur in *E. coli* and *Klebsiella* species via the production of expanded-spectrum β-lactamases (ESBLs) that are capable of hydrolyzing oxyimino-cephalosporins and monobactams (Pitout *et al.,* 2004). β-lactamase production is the most prevalent mechanism of resistance among Gram-negative bacteria (Garau *et al.,* 1997). Plasmid-mediated genes encoding ESBLs can be transferable within and between species, encouraging the spread of resistance among the Enterobacteriaceae (DiPersio and Dowzicky, 2007). However, broad-spectrum resistance to carbapenems and to fluorquinolones are today of the utmost significance. Ciprofloxacin is a very popular fluorquinolone for treatment of nosocomial infections, e.g. urinary tract infections. The frequent resistance to ciprofloxacin in Enterobacteriaceae is due to mutations in target genes. In recent years, a new resistance mechanism is increasingly observed (Pfeifer *et al.,* 2010). Carbapenem antibiotics are generally reserved for treatment of serious Gram-negative bacterial infections because these

antimicrobials typically retain activity (Anderson *et al.,* 2007; Centers for Disease Control and Prevention, 2009). Various mechanisms of carbapenem resistance exist (Bennett *et al.,* 2010; Paterson, 2006; Queenan and Bush, 2007).

Although the re-evaluation of older agents may be important (Falagas *et al.,* 2008), there is clearly a need for the development of new antimicrobial agents to keep in pace with the development and spread of drug resistance mechanisms among Gram-negative bacteria (Vergidis and Falagas, 2008). Finally, careful detection of resistant bacteria provides a fundamental basis for infection control measures and antimicrobial surveillance systems.

The objective of this study, is to investigate the incidence and prevalence of multidrug-resistant Gram-negative bacterial infection in hospitalized infants. This attempted to monitor the antimicrobial drug-resistance trends of pathogens in Mansoura University Children's Hospital (MUCH).

MATERIALS AND METHODS

Patient Samples

Sixty-five various clinical samples from urine (n = 28), blood (n = 23), cerebrospinal fluid (n = 4), stool (n = 8) and wound swabs (n = 2) were collected from the patients suffering from various bacterial infectious diseases. Specimens were cultured on different media at 37°C. The work was conducted at the Mansoura University Children's Hospital, during a four-month interval from July to October 2007. The data collected by daily surveillance of patients' charts included age, sex, ward, therapy and source of infection. The clinical features, laboratory data, causative organisms and antibiotic-administration schedule were collected and analyzed.

Collection and Processing of Samples

Urine Samples:

Early morning mid-stream urine samples were collected using sterile, wide mouthed glass bottles with screw cap tops. 1µl a loopful of the well mixed urine sample was inoculated into duplicate plates of Blood and MacConkey agar (Oxoid, Basingstoke, UK). All plates were then incubated at 37°C aerobically for 24 h. The plates were then examined macroscopically and microscopically for bacterial growth (Stamm *et al.,* 1982; Stark and Maki, 1984).

Blood Samples:

Before collecting the blood sample, skin was disinfected with 70% alcohol followed by 2% iodine tincture. Three milliliters of blood were obtained from peripheral vein puncture. A blood volume was injected into Bactec Peds Plus^{TM}/F culture vial for aerobic blood cultures. All blood samples were inoculated into the vials and processed using the Bactec Blood Culture system (Bactec; Becton Dickinson Diagnostic systems, USA) for incubation and periodic reading. A positive reading represented as a cloudiness in the vial indicates the presumptive presence of viable bacteria.

Positive vials were subcultured on nutrient agar base supplemented with 7% human blood and plates were incubated overnight at 37°C.

Cerebrospinal fluid (CSF) Samples:

The sterile lumbar puncture needle is inserted between the fourth and fifth lumbar vertebrae to a depth of 4–5cm. Approximately 5–10 ml of CSF are collected in two sterile tubes. Tube 1 will be used for visual inspection, microscopic and chemical examination. Tube 2 is used for bacterial culture. Portion of the CSF specimen inoculated on chocolate agar plate or tryptic soy broth. All media were incubated at 37°C in an atmosphere enriched with carbon dioxide for 3 days, with daily inspections.

Fecal Samples:

Fecal samples were collected in clean, wide-mouth tightly-fitted lid containers. Prepared a fecal suspension by suspending approximately 1 g of the stool sample in a tube containing 1 ml of sterile saline. Add three or more loopfuls of fecal suspension to selenite broth base (Biotec Laboratories Ltd, UK). Tubes were incubated for 18 hours. Subculture colonies by streaking a loopful of broth on MacConkey agar with crystal violet (Oxoid, Basingstoke, UK) as a general purpose medium.

Wounds Samples:

Wound samples first were decontaminated with surgical soap and 70% ethyl alcohol, after the wound is washed well with sterile saline and dried. Swabs are used most commonly and placed immediately into an appropriate transport container. Swabs were inoculated on nutrient agar and plates were incubated at 37°C for 24 h.

Microbial Identification

Identification of bacterial pathogens was conducted on the basis of gram reactions and morphology of colony. Subsequently, Gram-negative bacteria were identified using routine standard biochemical assays including triple sugar iron (TSI), urease, indol and citrate. A Microscan Gram Negative Breakpoint Combo Identification (NID) panels type 12 (Siemens HealthCare Diagnostics, formerly Dade Behring, USA) were used to confirm the identification of Gram-negative facultative bacilli according to the manufacturer's manual system.

Inocula Preparation

The Prompt inoculation wand is held perpendicular to the surface of the agar and three isolated colonies are touched with the wand tip. The collar is removed and the wand is placed in the prompt bottle after the bottle top is snapped off. The bottle is shaken vigorously to create a suspension of the bacteria in the 30 ml of the stabilized aqueous Pluronic-D. The suspension is poured into the seed tray to inoculate the MicroScan panel.

Incubation

A clean cover tray is placed on top of each panel to prevent evaporation. The panels are incubated for 16-20 hours at 35°C in a non-CO_2 incubator.

Biochemical Tests

In each Microscan NID type 12, several biochemical tests were performed. These included carbohydrate fermentation tests using raffinose, sucrose, sorbitol, arabinose, inositol, rhamnose, and glucose. Also, susceptibilities to kanamycin, and colistin were tested. In addition, fluorogenic substrate tests were performed for the detection of various bacterial

enzymes. In these tests, different substrates linked chemically to fluorophores were used (e.g., tryptophan deaminase, urease, and galactosidase). Other specific tests were also used, such as Voges Proskauer, nitrate reduction, hydrogen sulfide production, and utilization of citrate.

Antimicrobial Susceptibility Testing

Both automated and manual methods were used to detect the antimicrobial susceptibility pattern of the isolates. The Microscan Gram Negative Breakpoint Combo panel (NBPC) type 12 automated system was used for 31 antimicrobial susceptibility testing of Gram-negative isolates. Prompt Inoculation System was used to inoculate the panels. Incubation and reading of the panels were performed in the MicroScan WalkAway Microbiology System (Siemens HealthCare Diagnostics, formerly Dade Behring, USA).

The Kirby-Bauer technique was used to detect resistant Gram-negative isolates. Discs of several antimicrobial disks were placed on the surface of Muller-Hinton agar plates (Oxoid, Basingstoke, UK) followed by incubation at 37°C (Bauer and Kirby, 1966). Reading of the plates was carried out after 24 hours using transmitted light by looking carefully for any growth within the zone of inhibition (Cafferkey, 1992). Depending on the inhibition zone diameter produced, the effect is classified as sensible (S), intermediate (I) or resistant (R). Susceptibility of the transconjugants to the antibiotics was determined by standard disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2006). The following antibiotic disks (Oxoid, England) were used in susceptibility testing of the transconjugants: trimetoprim/sulphamethoxazole (1.25 µg/23.75 µg), gentamicine (10 µg), cefotaxime (30 µg), levofloxacin (5 µg), piperacillin (100 µg), imipenem (10 µg), ciprofloxacine (5 µg), nitrofurantoin (300 µg), amikacine (30 µg) and tobramycin (10 µg).

RESULTS AND DISCUSSION

Infections caused by Gram-negative bacteria have continued to be a major problem for hospitalized patients (Neu, 1985). Hospitalized children are at increased risk for colonization and infection by multidrug-resistant organisms (Anderson *et al.,* 2008; Bradford, 2001). Moreover, colonization with multidrug-resistant organisms, and specifically with multidrug-resistant Enterobacteriaceae (MDRE), put patients at increased risk for healthcare-acquired infections (Singh *et al.,* 2002). A total of 65 Gram-negative isolates were collected from 65 patients with a median age of 8 months and an average stayed in the hospital of 18 days. Gram-negative pathogens isolated from different clinical specimens of patients were mainly of enterobacterial species (Table 1). Bloodstream infections (BSIs) caused by Gram-negative (GN) pathogens are re-emerging; in the last few years (Rodriguez-Créixems *et al.,* 2008). Gram-negative bacteremia is frequently found in clinical practice among all age groups (Stryjewski and Boucher, 2009). The sources for Gram-negative septicaemia are mainly urinary tract infection (UTI), and gastro-intestinal tract infection (GITI) (Marschall *et al.,* 2008). Bacteremia due to *Klebsiella* (Levy *et al.,* 1996)*, E. coli, Enterobacter and Pseudomonas* has

been reported in pediatric patients (Ford-Jones *et al.,* 1989). Andresen *et al.* (1994) showed relative importance of *Enterobacter* bacteremia in pediatric patients. Thus organisms causing septicaemia differ from place to place. Gram-negative bacilli cause the overwhelming majority of UTIs (Ackermann and Monroe, 1996). Although urinary tract infections (UTIs) are one of the serious bacterial infections encountered in children, there are only a limited number of studies examining resistance of urinary tract pathogens in the pediatric population (Fanos and Khoory, 1999). Of 65 patients, 28 (43%) were diagnosed with urinary tract infections (UTIs) and 23 (35.4%) with bloodstream infection (BSI). The main isolated Gram-negative bacteria from urine were *Escherichia coli* (25%) and *Enterobacter aerogenes* (14.3%). UTI accounts for a significant part of the work load in clinical microbiology laboratories and enteric bacteria (in particular, *Escherichia coli*) remain the most frequent cause of UTI (Schappert, 1999), although the distribution of pathogens that cause UTI is changing (Ojiegbe and Nworie, 2000). As shown in Table 2, the main isolated Gram-negative bacteria from blood was *Klebsiella pneumoniae* (30.4%). The predominant isolated Gram-negative bacteria from all specimens were *K. pneumoniae* (15.4%; 10 out of 65 total Gram-negative isolates) followed by *E. coli* (12.3%), *E. aerogenes* (10.8%), *E. cloacae* (9.2%) and *Serratia fonticola* (9.2%; Table 2).

Table 3 shows the prevalence and distribution of clinical isolates of gram-negative bacteria emerging in different hospital units. *Enterobacter* spp*.* (including *E. aerogenes, E. cloacae, C. lapagei* or *C. species 3*) were the most commonly isolated pathogens followed by *K. pneumoniae*, *Serratia* spp*.* and *E. coli*.

Age and Sex Incidence

Urinary tract infection occurring from the neonate to the geriatric age group (Raju and Tiwari, 2004). The incidence of UTI is greater in females as compared to males (Schaeffer *et al.,* 2001). Gram-negative bacteria is a major cause of sepsis in neonates, contributing substantially to the mortality and morbidity (Daoud *et al.,* 1995). The incidence rate of Gram-negative bacteria increased within the age brackets of 1 month- < 1 year in both females and males (Fig. 1). Comparatively, there were more cases in females than males. *K. pneumoniae* was the most common cause of Gram-negative infection and accounted for 15% of episodes. The distribution of pathogens causing gram-negative infection by gender is shown in Table 4.

The incidence rate of *E. coli* was higher in females than in males and the incidence rate of *E. cloacae* was higher in males than in females (Table 4).

Table 1. Identification of gram-negative bacteria isolated.

Identification	Number of Isolates	Percentage
Enterobacterial bacteria	53	81.54
Non-fermenting bacteria	9	13.85
Other	3	4.61
Total	**65**	**100**

Table 2: Distribution of gram-negative bacteria isolated in various clinical specimens.

Isolated Organisms	Urine		Blood		CSF**		Stool		WS**		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%*
Klebsiella pneumoniae	2	7.14	7	30.4	–		–		1	50	10	15.4
Escherichia coli	7	25	–		–		1	12.5	–		8	12.3
Enterobacter aerogenes	4	14.3	1	4.35	–		2	25	–		7	10.8
Enterobacter cloacae	3	10.7	2	8.7	–		1	12.5	–		6	9.2
Serratia fonticola	3	10.7	2	8.7	–		–		1	50	6	9.2
C. freundii complex	3	10.7	–		–		2	25	–		5	7.7
Kluyvera ascorbata	2	7.14	–		2	50	–		–		4	6.2
Ac. baumann/haem	–		3	13.04	–		–		–		3	4.6
Pseudomonas aeruginosa	–		2	8.7	–		–		–		2	3.1
Ps. fluorescens/putida	1	3.57	1	4.35	–		–		–		2	3.1
Vibrio alginolyticus	–		–		1	25	1	12.5	–		2	3.1
Vibrio damsela	–		–		–		1	12.5	–		1	1.53
Cedecea lapagei	–		1	4.35	–		–		–		1	1.53
Cedecea species 3	–		1	4.35	–		–		–		1	1.53
Proteus mirabilis	1	3.57	–		–		–		–		1	1.53
Providencia stuartii	–		–		1	25	–		–		1	1.53
Serratia marcescens	–		1	4.35	–		–		–		1	1.53
Serratia plymuthica	1	3.57	–		–		–		–		1	1.53
Serratia liquefaciens	1	3.57	–		–		–		–		1	1.53
S. maltophilia	–		1	4.35	–		–		–		1	1.53
Acinetobacter lwoffii	–		1	4.35	–		–		–		1	1.53
Total	28	43	23	35.4	4	6.2	8	12.3	2	3.1	65	100

*** Percentage refers to the proportion of all episodes.**

**** CSF, cerebrospinal fluid; WS, wound swab; *C. freundii complex, Citrobacter freundii complex*; *Ac. baumann/haem*, *Acinetobacter baumannii/haemolyticus*; *Ps. fluorescens/putida*, *Pseudomonas fluorescens/putida*; *S. maltophilia*, *Stenotrophomonas maltophilia*.**

Table 3: Prevalence and distribution of clinical isolates of gram-negative bacteria emerging in different units of the hospital.

Isolate	Gen.* & Hae.	End	Car	Gast	Neo	ICU	Neu	Neph	Infe & Nut	Alle & imm	T
Klebsiella		2	2		3		1			2	10
Enteroba-cter	2	2	3	2	3		2		1		15
Serratia			2		6	1					9
Escherichia		1	2	2				1	1	1	8
Citrobacter	1	1		1				2			5
Kluyvera	1						2	1			4
Acinetobacter				1	1			1		1	4
Pseudomonas		1		2		1					4
Vibrio			1						1	1	3
Proteus										1	1
Providencia				1							1
Stenotrophomonas			1								1
Total	4	7	11	9	13	2	5	5	3	6	65

*** Gen. & Hae., Pediatric Genetics & Haematology; End, Endocrinology; Car, Cardiology; Gast, Gastroenterology; Neo, Neonatology; ICU, Intensive-Care Unit; Neu, Neurology; Neph, Nephrology; Infe & Nut, Infection & Nutrition; Alle & Imm, Allergy & Immunology; T, Total.**

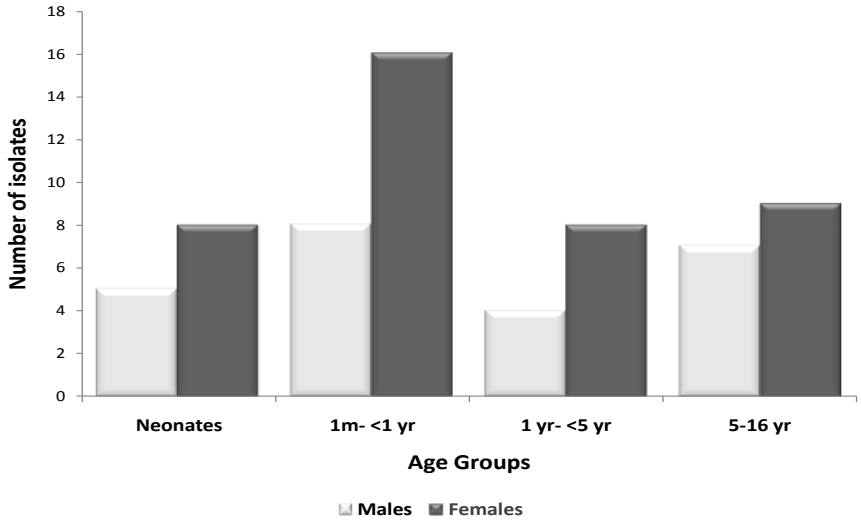

Figure 1. Incidence rate of identified gram-negative bacteria by age and gender.

Table 4. Pathogen distribution of identified gram-negative bacteria in relation to gender.

Pathogen	Gender		Total N (%)*
	Female	Male	
K. pneumoniae	7	3	**10 (15.4%)**
E. aerogenes	6	1	**7 (10.8%)**
E. cloacae	1	5	**6 (9.2%)**
C. lapagei	1	0	**1 (1.5%)**
C. species 3	0	1	**1 (1.5%)**
S. fonticola	4	2	**6 (9.2%)**
S. liquefaciens	1	0	**1 (1.5%)**
S. marcescens	0	1	**1 (1.5%)**
S. plymuthica	1	0	**1(1.5%)**
E. coli	8	0	**8 (12.3%)**
C. freundii cplx	3	2	**5 (7.7%)**
K. ascorbata	1	3	**4 (6.15%)**
Ac. baumannii/haem	1	2	**3 (4.6%)**
A. lwoffii	0	1	**1 (1.5%)**
P. aeruginosa	2	0	**2 (3.1%)**
Ps. fluor/putida	1	1	**2 (3.1%)**
Other	4	2	**6 (9.2%)**
Total	**41**	**24**	**65 (100%)**

*** N, number (percentage refers to the proportion of total episode).**

Similarly, *K. pneumoniae* was the most common causative organism in all age groups, but it was more predominant in neonates born. Incidence of *Acinetobacter* infection in neonates has been reported by Horrevorts *et al.* (1995) and is a major problem in neonatal intensive care unit (Bernards *et al.,* 1997).

K. pneumoniae caused 8–31% of Gram-negative infection in patients <1 year old compared to 6–25% in patients ≥1 year old (Table 5). *E. coli* was more common in older children; while *E. coli* caused 13–19% of Gram-negative infection in patients ≥1 month old, it did not contribute to Gram-negative infection in patients <1 month of age. *P. aeruginosa* was identified in 8% of Gram-negative infection in children between the age of 1 month to < 1 year.

Table 5: Pathogen distribution of identified gram-negative bacteria by age group.

Rank	Age group			
	0-28 days N = 13	**1 month- <1 year N = 24**	**1 year- <5 years N = 12**	**5-16 years N = 16**
1	*K. pneumoniae*(31)	*E. aerogenes* (17)	*K. pneumoniae*(25)	*S. fonticola* (25)
2	*E. cloacae* (15)	*E. coli* (13)	*E. coli* (17)	*E. coli* (19)
3	*S. marcescens* (8)	*K. pneumoniae* (8)	*C. freundii cplx*(17)	*E. cloacae* (19)
4	Other (46)	*C. freundii cplx* (8)	*C. lapagei* (8)	*E. aerogenes* (13)
5		*K. ascorbata* (8)	Other (33)	*K. pneumoniae* (6)
6		*P. aeruginosa* (8)		Other (19)
7		Other (37)		

Data are displayed as causative organism (percentage of the total in the corresponding age group column).

In Vitro Antimicrobial Resistance Rates

Numerous studies have shown an association between the development of resistance in Gram-negative bacteria (GNB) and increases in mortality, length of stay (LOS) in hospital, and cost of care (Cosgrove, 2006; Tumbarello *et al.,* 2006). This is especially the case among *Enterobacter* spp. (Cosgrove *et al.,* 2002), *E. coli* (extended-spectrum β-lactamase [ESBL] producers) and *Klebsiella* spp. (ESBL) (Cosgrove, 2006; Paterson *et al.,* 2005). The antimicrobial susceptibility pattern obtained using the Microscan Gram Negative Breakpoint Combo panel (NBPC) type 12 automated system is shown in Table 6. According to the results, a high resistance rate to different common antibiotics is shown by the isolated strains. The highest resistance rates correspond to ampicillin, followed by cefuroxime and trimethoprim/sulfamethoxazole. The most active antimicrobial agents were imipenem (IPM) and gatifloxacin (GAT). *K. pneumoniae* was resistant to cephalosporins and aminoglycosides; moderate resistance to trimethoprim/sulfamethoxazole (SXT). *E. coli* were susceptible to amikacin (AK), cefoxitin (FOX), nitrofurantoin (F) and relatively less sensitive to penicillins, trimethoprim (TMP) and tetracycline (TE). Two of the *E. aerogenes* isolates were resistant to all antibiotic groups, and the rest showed very limited antimicrobial susceptibility, mostly only to carbapenems, nitrofurantoin and piperacillin/tazobactam (TZP). Cefuroxime (CXM) and tobramycin (TOB) were poorly effective with *E. cloacae*. In recent years, *Enterobacter cloacae* has emerged as an important pathogen that is intrinsically resistant to ampicillin and narrow-spectrum cephalosporins (Treviño *et al.,* 2009). One *S. fonticola* isolate showed no susceptibility to any of the tested antibiotics.

Many Enterobacteriaceae are resistant to all antibiotics except carbapenems (Livermore, 2004); therefore, antibiotic development remains vital to keep ahead of resistance. Fluoroquinolones have proven to be highly effective broad-spectrum agents for a variety of infections, especially those caused by Gram-negative bacilli. The fluoroquinolone levofloxacin has greater activity than ciprofloxacin against Gram-negative pathogens such as *E. coli*, *K. pneumoniae* and *P. aeruginosa* (Davis and Bryson, 1994). In this study, Ciprofloxacin, gatifloxacin, levofloxacin and norfloxacin displayed the highest activity against Enterobacteriaceae. This result is a consequence of very low consumption of fluoroquinolones in pediatric.

Table 6. Antibiotic resistance from predominant gram-negative bacteria, using automated method.

Antibiotic	%				
	K. pneumoniae	***E. coli***	***E. aerogenes***	***E. cloacae***	***S. fonticola***
Amikacin	30	0	57.1	0	83.3
Ampicillin	100	87.5	100	100	100
Cefepime	30	12.5	42.9	33.3	50
Cefotaxime	70	50	100	33.3	83.3
Cefotetan	10	12.5	0	50	66.7
Ceftazidime	70	25	85.7	50	83.3
Ceftriaxone	80	50	100	33.3	83.3
Cefuroxime	80	62.5	100	66.7	100
Ciprofloxacin	20	25	57.1	0	66.7
Cefoperazone	30	62.5	57.1	0	16.7
Cefoxitin	10	0	28.6	50	33.3
Cefpodoxime	40	50	57.1	50	16.7
Ceftizoxime	30	37.5	57.1	33.3	33.3
Gatifloxacin	0	0	28.6	0	0
Gentamicin	80	37.5	85.7	33.3	83.3
Imipenem	10	0	14.3	0	33.3
Levofloxacin	0	25	42.9	0	33.3
Meropenem	10	12.5	14.3	0	16.7
Nitrofurantoin	10	0	14.3	33.3	83.3
Pip/Tazo*	20	12.5	14.3	33.3	33.3
Ticar/K Clav*	40	12.5	100	33.3	100
Tobramycin	80	37.5	71.4	66.7	83.3
Trimeth/Sulfa*	50	87.5	100	50	100
Aztreonam	30	25	57.1	16.7	33.3
Mezlocillin	40	75	57.1	0	16.7
Netilmicin	40	12.5	42.9	0	33.3
Norfloxacin	0	25	42.9	0	16.7
Piperacillin	40	75	57.1	0	33.3
Tetracycline	30	75	57.1	33.3	33.3
Ticarcillin	40	75	57.1	16.7	33.3
Trimethoprim	30	75	57.1	50	16.7

*** Pip/Tazo, Piperacillin/Tazobactam; Ticar/K Clav, Ticarcillin/K Clavulanate; Trimeth/Sulfa, Trimethoprim/Sulfamethoxazole.**

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توزيع عدوى البكتريا السالبة بصبغة الجرام المقاومة للعديد من المضادات الحيوية في الأطفالِ المُدخَلون المستشفى

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*****قسم النبات - كلية العلوم بدمياط - جامعة المنصورة- دمياط – مصر.**

تم تجميع خمسة وستون عزلة من البكتريا السالبة لصبغة الجرام من عينات مختلفة (دم, بول, براز, السائل الشوكى, مسحات الجرح) من مستشفى الأطفال الجامعى بالمنصورة, وعرفت هذة المعزولات كالأتى: كليبسيلا بنيمونيا (10 عزلات), إشيريتشيا كولاي (8 عزلات), إنتروباكتر إروجينيز (7 عزلات), إنتروباكتر كلوكاى (6 عزلات), سيراتيا فونتكولا (6 عزلات), سيتروباكتر فروندى المركب (5 عزلات), كليفيرا أسكورباتا (4 عزلات), أسينيتوباكتر بومانني/هيموليتكس (3 عزلات), بسيودوموناس إروجنوسا (2 عزلة), بسيودوموناس فلورسينس بوتدا (2 عزلة), فيبريو ألجنوليتكس (2 عزلة), فيبريو دامسيلا (1 عزلة), سيديسي لاباجى (1 عزلة), سيديسي نوع 3 (1 عزلة), بروتيوس مرابيليس (1 عزلة), بروفيدينسيا ستوارتى (1 عزلة), سيراتيا مارسيسينس (1عزلة), سيراتيا بلموسيكا (1 عزلة), سيراتيا ليكوفاكينس(1 عزلة), ستينوتروفوموناس مالتوفيليا (1 عزلة), وأسينيتوباكتر لوفى (1 عزلة).وقد تم أختبار واحد وثلاثون من المضادات الميكروبية ضد هذة العزلات البكترية. وكان جاتيفلوكساسين, إبينيم, ميروبينيم أكثر المضاداتَ فاعليةَ ضدّ البكتيريا المَعْزُولةِ, بينما الأمبيسيلين, السيفوتاكسيم, السيفتازيديم, السيفتراكسون, السيفوروكسيم, جينتاميسين, توبراميسين تَبَيَّن بأن لهم تأثيرَ منخفضَ ضدّ البكتيريا المَعْزُولة.

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