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Determination of Antimicrobial Resistance of *Enterobacteriaceae* in Patients with Community Acquired Urinary Tract Infections

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Abstract: This study was conducted in Port Sudan city, Red Sea state during the period from January to June 2017 to investigate antimicrobial resistance of *Enterobacteriaceae* isolated from patients suffering from community acquired urinary tract infections. One hundred and fifty urine specimens were collected from patients attended Port Sudan Teaching Hospital. The specimens were cultured on blood agar and Mac Conkey's agar and Cystine Lysine Electrolyte deficient (CLED) agar for primary isolation of pathogens. Identification of the isolates was done by colonial morphology, Gram stain and Routine biochemical tests. Modified Kirby-Bauer disc diffusion method was adopted to determine the resistance rate of *Enterobacteriaceae* to Imipenem, Ciprofloxacin, Chloramphenicol, Amikacin, Piperacillin, Tetracycline, Ceftazidime and Ticarcillin. Out one hundred and fifty urine specimens examined *Enterobacteriaceae* was detected in only 49(32.6%) specimens. The results revealed that the antimicrobial resistance of *Enterobacteriaceae* was as follows: Imipenem (6.1%), Ciprofloxacin (32.6%), Chloramphenicol (48.9%), Amikacin (61.2%), Piperacillin (79.5%), Tetracycline (83.6%), Ceftazidime (89.7%) and Ticarcillin (91.8%). Females were more affected than males (60%) and young adults were more affected than other age groups. Imipenem represented the least sensitive antimicrobial agent (6.1%), while Ticarcillin showed the highest resistance rate.

Keywords: Enterobacteriaceae, Urinary Tract Infections, CLED, Port Sudan

1. Introduction

Definition: Any infection acquired in the community, that is, contrasted with those acquired in a health care facility (CROSS INFECTION). An infection would be classified as community-acquired if the patient had not recently been in a health care facility or been in contact with someone who had been recently in a health care facile.

Urinary tract infections (UTIs) are one of the most common infectious diseases, and nearly 10% of people will experience a UTI during their life time. The infections may be symptomatic or asymptomatic, and types of infection can result in serious sequelae if left untreated 4 M [1].

Although several different micro organisms can cause UTIs, including fungi and viruses, bacteria are the major causative organisms and are responsible for more than 95% of UTI cases [1].

The increase in the number of resistant and multiresistant (resistant to two and more antimicrobials) strains of bacteria is a major concern of health officials worldwide. Recently, bacterial resistance arising through the production of extended – spectrum beta – lactamases (ESBLs) has been recognized as a worldwide therapeutic problem [2]. The mechanisms of this resistance are often complex, and include production of β- lactamases unregulated efflux pumps, and target site mutations [3].

In Gram–negative pathogens, β – lactamases production remains the most important contributing factor to β-lactam

resistance.1 DM Livermore, Bacterial resistance: origins, epidemiology, and impact, Clin Infect Dis 36 (suppl 1) (2003), pp. S11–S23. Full Text via CrossRef | View Record in Scopus | Cited By in Scopus (147) lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, which results in ineffective compounds. One group of β lactamases; extended - spectrum β lactamases (ESBLs); have the ability to hydrolyse and cause resistance to various types of the newer β-lactam antibiotics, including the expanded-spectrum (or third-generation) cephalosporins (e.g., cefotaxime, ceftriaxone and ceftazidime) and monobactams (e.g., aztreonam), but not the cephamycins (e.g., cefoxitin and cefotetan) and carbapenems (e.g., imipenem, meropenem, and ertapenem). Organisms that produce ESBL remain an important reason for therapy failure with cephalosporins and have serious consequences for infection control. That clinical microbiology laboratories detect and report ESBL – producing organisms is therefore important. Most ESBLs can be divided into three groups: TEM, SHV, and CTX-M types [4].

Wild type strains of are susceptible to all penicillins and cephalosporins. However, since 1990, a progressive increase of β-lactam. resistance, mediated by the production of acquired β-lactamases, has occurred in this species. [3] A. Endimiani, F. Luzzaro, G. Brigante, M. Perilli, G. Lombardi and G. Amicosante et al., Proteus mirabilis bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum β-Lactamases, Antimicrob Agents Chemother 49 (2005), pp. 2598–2605. View Record in Scopus | Cited By in Scopus Various ESBLs, such as TEM, CTX-M, PER, and VEB – type ESBLs, have been described in P. mirabilis in separate geographic areas [5]. Available therapeutic options for the treatment of these antimicrobial -resistant organisms are severely limited because these organisms frequently display a multidrug – resistant phenotype [6].

Treatment of UTI cases is often started empirically. Therapy is based on information determined from the antimicrobial resistance pattern of the urinary pathogens. However, because of the evolving and continuing antibiotic resistance phenomenon, regular monitoring of resistance patterns is necessary to improve guidelines for empirical antibiotic therapy.

2. Materials and Method

2.1. Study Approach

Study approach is to identify and isolate the possible causative organisms and the characterization of isolated strains using bio typing and anti bio gram.

2.1.1. Study Type and Design
Cross-sectional descriptive study.

2.1.2. Study Area
This work was carried out in Red Sea state. Urine collection and patients data were done in Port Sudan Teaching Hospital (PTH), Sea Port Corporation Hospital, Prince Osman Digina Hospital. The laboratory investigation was done in the Port Sudan Alahlia college.

2.1.3. Target Population
Patients suffering from UTI signs and symptoms that attended the above mentioned hospitals were included in this study. The patients age varied from 2 years to 75 years.

2.1.4. Data Collection
Data were collected using structural interviewing questionnaire.

2.2. Methodology

2.2.1. Sample Size and Collection
According to standard method all specimen were examine to detect, and identify pathogens or their products using: microscopic examination of specimen to detect their motility, morphology and staining reaction. Also culture techniques to isolate pathogens in pure form and to identify them then test their antibiotic sensitivity and biochemical.

2.2.2. Sampling
Samples for community acquired UTI to investigate and diagnosis of microbial diseases (urine specimen).

Urine specimens were collected in case of urinary tract infection as following. A mid stream urine is obtained in a sterile container after cleaning the external genitalia with tap water and drying. Samples should reach the laboratory within one hour after voiding or kept refrigerated at 4c^o to avoid multiplication of bacteria in urine then we do appropriate test to identify and isolate the causative organisms.

2.3. Cultivation of Specimens

Urine specimens were inoculated onto CLED medium by using sterile loop, then incubated aerobically at 37c^o over night.

2.4. Examination of Bacterial Growth

The primary culture on CLED medium that showed significant growth was examined for fermentation. The morphological character, size, shape, colour were observed and recorded.

2.5. Interpretation of Culture Growth

The culture growth obtained was interpreted as significant ($>10^5$CFU/ml). Cultured of less than (103CFU/ ml) of urine was considered insignificant, while culture with no growth were considered negative. Significant cultures were further investigated.

2.6. Purification of Bacterial Growth

The isolates were streaked onto Nutrient agar and incubated overnight at 37°C. The resultant growth was checked for purity and stored in Bijou bottle for further investigation.

2.7. Identification of the Isolated Bacteria

2.7.1. Colonial Morphology

Colonial characteristic were observed on CLED medium after overnight incubation isolated organisms were growth on CLED medium.

2.7.2. Biochemical Tests (Conventional Test)

These are called biochemical tests because they are tests which identify the bacteria on the basis of the presence of certain enzymes and other biochemical properties.

a. Oxidase Test

In this test organism that produce the enzyme Cytochrome Oxidase oxidize phenylenediamine to a deep purple color. Oxidase reagent disc were placed on sterile Petri plates and rubbed with colony of the test organism using a piece of stick, and looked for a red-purple color with in 20 min.

b. Kliglar Iron Agar

this medium was originally designed as a multi-test medium. It provides a low degree of sensitivity for H2S production (often required when differentiating members of the (Enterobacteriaceae). The medium is now used principally as a standard test for H2S. One disadvantage of multitest media is that chemical interaction-in this case acid production from fermentable sucrose-may inhibit blackening of the iron indicator. Some Citrobacter and Proteus species have this ability. KIA agar should be used in conjunction with a urease test to eliminate Proteus spp when screening for Salmonellae.

c. Citrate Utilization Test

This is for the ability of on organism to utilize citrate as the sole carbon and energy source for growth and an ammonium salt as sole source of nitrogen.

d. Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Indole is then tested for by a colorimetric reaction with p- dimethylaminobenzaldehyde. Add 0.5ml kovacs reagent to see result.

e. Urease Test

Bacteria, particularly those growing naturally in an environment exposed to urine, may decompose urea by means of the enzyme urease The occurrence of this enzyme can be tested for by growing the organism in the presence of urea and testing for alkali production by means of a suitable PH indicator. An alternative method is to test for the production of ammonia from urea by means Nesslers reagent.

f. Motility Test

In semi solid agar media, motile bacteria (Swarm) and give a diffuse spreading growth that is easily recognized by the naked eye. Motility may thus be detected more easily than by the microscopical method.

2.8. Antimicrobial Sensitivity Test

All isolated microorganisms were subjected to antimicrobial sensitivity test using Modified Kirby-Bauer disc diffusion method.

3. Results

One hundred and fifty urine specimens were collected from patients suffering from UTI in the period from January 2017 to june 2017. The patients constitute 60 (40%) males and 90 (60%) females (Table 1). The age of the patients ranged from two years to 75 years. Out of 150 patients (0%) were infants, 8 (5.3%) were children, 68 (45.3%) were young adult, 42 (28%) were adults and 32 (21.3%) were elderly (Table 2). 120 (54%) of specimens showed bacterial growth when cultured on Blood, MacConkey agar and CLED. Among these 32 (21.3%) were lactose fermenters (LF) and the rest 17 (11.3%) were non-lactose fermenters (Table 3).

***Table 1.** Distribution of specimens according to patients gender.*

Gender	No of specimen	%
Male	60	40
Female	90	60
Total	150	100

***Table 2.** Distribution of specimens according to patients age group.*

Total Number of specimens	Significant growth (%)		Insignificant growth(%)
150 specimens	49 (32.6%)		101(67.3%)
	NLF(%)	LF(%)	
	8%17(11.3%)	32(21.3%)	

***Table 3.** Significant and insignificant growth on MacConkey`s agar.*

Age group	No. of specimens
0 – 5 (Infant)	0
5 – 12 (Children)	8 (5.3%)
16-30 (Young adult)	68 (45.3%)
Elder (more than 46)	32(21.3%)

***Table 4.** Activity of antibiotic on Enterobaciaceae.*

Activity of antibiotic	IPM	CIP	C	AK	PI	TE	TI	CAZ
Sensitive	44 (89)	29 (59)	20 (40.)	18 (36.)	8 (16.)	5 (10.)	4 (8.1)	5 (10.)
Resistance	16 (6.)	24 (3)	24 (48)	30 (61)	39 (79)	41 (83)	45 (91.)	44 (89.7)

Key:
(IPM) Imipenem, (CIP) ciprofloxacin, (C) chloramphenicol, (AK) amikacim, (PI) piperacllin (TE) tetracucline, (CAZ) Ceftazitim (Ti) Ticarcillin.

4. Discussion and Conclusion

4.1. Discussion

This study was conducted to determine antimicrobials resistance profile of *Enterobacteriaceae* isolated. In this study out of one hundred and fifty urine specimens investigated, 49(32.6%) showed significant bacterial growth, while 101(67.3%) showed no growth and this result nearly similar with result obtained by Dr. Ummey Shahnaz Parvin [7], and disagree with the result was obtained by Muktikesh Dash *et al*, [8].

The present study females were more susceptible to infection 90 (60%) while male less susceptible 60 (40%) this result was agree to result obtained by Samiah HS Al-Mijalli *et al* [9].

The current study show that the, 68 (45.3%) were young adult were most effect this result disagree with the result was obtained by Mohammed Akram, *et., al* [5]. Among these *Escherichia coli* 25 (51%), *Klebsiella spp* 31(26%), *proteus spp 8(16%), Citrobacter sp2(4%), Salmonella spp 1(2.5%).* This result agree with result was obtained by Muktikesh Dash *et al*, [8],

In vitro activities of antimicrobial agents showed that the resistance rate of *Enterobacteriaceae* to ticarcillin (91.2%) This result is disagrees with the result obtained by Nijssen *et al*, [10].

In present study the imipenem show highly sensitivity for UTIs and this result nearly similar with the result was obtained by Samiah HS Al-Mijall *et al*,. [9], and ciprofloxacin was show as 2nd effective antimicrobial against urinary tract infection caused by *Enterobacteriaceae* this result agree with the result was obtained by J Hosp Infect. [11].

4.2. Conclusion

The study concluded that:
i. UTIs in a young adults, children and elder were more than infant.
ii. Most effective antibiotics for *Enterobacteriaceae* was imipenem and ciprofloxacin in due to low resistance.
iii. Chloramphenicol, amikacim and piperacllin are the second choice of antimicrobial therapy with resistance rate.
iv. Tetracycline, ceftazitim and Ticarcillin is less effective antimicrobial agents due to high resistance rate.

Recommendations

i. All urine specimens seen on routine work should investigated for culture and susceptibility tests.
ii. Introduction of the API 20 E for *Enterobacteriaceae* isolation is necessary in all Microbiology Laboratories.
iii. Establishment of antimicrobial policies and treatment guidelines are of major importance.

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