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Research Article

Phenotypic Detection of Extended-Spectrum B- Lactamase Producing *Escherichia coli* and *Klebsiella pneumoniae* from a Vaginal Swab of Pregnant women in Khartoum State

Babbiker Mohammed Taher Gorish*

Lecture of Medical Microbiology Omdurman Islamic University, Sudan

***Corresponding Author:** Babbiker Mohammed Taher Gorish, Lecture of Medical Microbiology Omdurman Islamic University, Sudan.

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Abstract

E. coli and *K. pneuomniae* are best Known cause of neonatal meningitis. Antimicrobial sensitivity test of both bacterial isolates were extensively studied in Sudan. This study was aimed to determine the frequency of ESBL producing *E. coli* and *K. pneuomniae* which isolated from a vaginal swab of pregnant women in Khartoum state. During the period from November 2018 to March 2019 a total of 300 vaginal swabs were collected. These swabs were screened for the presence of *E. coli* and *K. pneuomniae* by cultivation in Macconkey agar. The isolated organisms were identified by using a biochemical test set of IMVIC and KIA agar. Both isolates were screened for ESBL by using phenotypic double disc synergy test through cefotaxime (30 µg) or ceftazidime (30 µg) disks with clavulanate (10 µg). Other antibiotic potency against these isolates were also detected by using antimicrobial sensitivity test according to CLSI recommended method. Out of 300 vaginal swabs cultivated 67 (22.3%) were showed a growth of which 28 (41.8%) were *E. coli,* 9 (13.4%) were *K. pneuomniae* and 30 (44.8%) were other bacteria. The frequency of ESBL among *E. coli* isolates was 7 out of 28 (25%) while only 2 (22.2%) *K. pneuomniae* isolates out of 9 were showed appositive synergy test. Ciprofloxacin was the most potent antibiotic against ESBL Producing strain followed by Imipenem, Gentamycin, Amikacin, Nitrofurantoin, Sulfamethoxazole, and Chloramphenicol. We conclude that there is a moderate prevalence of ESBL producing *E. coli* and *K. pneuomniae* isolates among vaginal isolates that may lead to neonatal meningitis which could be difficult to be treated.

Keywords: *E. coli; K. pneumniae*; ESBL; Synergy Test; Vaginal Swab

Background

Extended-spectrum B- lactamase (ESBL) are enzymes produced by many Gram-negative Enterobacteriaceae [1]. The ESBL are plasmid-mediated enzymes with the capability to hydrolyze and inactivate broad spectrum of β-Lactam antimicrobials, including third-generation cephalosporins, penicillins, and aztreonam; but are inhibited by clavulanic acid [2]. Although most of the ESBLs are mutants of the TEM and the SHV enzymes, the CTX-M type beta-lactamases have become more important. The CTX-M type of enzyme constitutes a distinct lineage of the molecular class A β lactamases which are a rapidly growing group [3]. ESBL-producing organisms are often also able to reduce the susceptibility of other non-β-lactamase antimicrobial classes, such as aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, tetracyclines, and nitrofurantoin; thus, leaving a limited range of therapeutic agents [4].

Neonatal meningitis is the term used to describe meningitis that occurs in the first 28 days of life. Many different organisms can cause neonatal meningitis, broadly grouped as bacteria, viruses, and fungi. *E. coli* is the most common cause of Gram-negative neonatal bacterial meningitis and septicemia followed by *K. pneunoniae.* In developing countries, the mortality from neonatal meningitis is estimated to be 40-58%, compared to 10% in developed countries [5].

When the microorganisms acquired from the mother before or during birth. bacteria overcome the body's defenses and cause infection. In this process, the bacteria may spread through the bloodstream to the meninges and cause meningitis. When the bacteria infect the meninges, tiny blood vessels in the membranes are damaged. This allows the bacteria to break through and infect the cerebrospinal fluid. The meninges then become inflamed, increas-

ing pressure around the brain which can cause nerve damage and specific symptoms associated with meningitis [6].

Neonatal meningitis is much more common in developing countries. Neonatal meningitis ranges from 4.8 per 10,000 live births in Hong Kong to 24 per 10,000 live births in Kuwait. In Africa and South Asia, figures ranging from 8.0 to 61 per 10,000 live births are found. It is expected that these numbers are lower than reality due to the difficulty of diagnosing and the healthcare available to underdeveloped countries in Asia and Africa [7].

Neonatal infections are responsible for about 1.6 million deaths every year in developing countries. Sepsis and meningitis are considered the most common causes of these deaths [8]. In developed countries, Streptococcus agalactiae (GBS) and Escherichia coli were the most common pathogens isolated in severe cases [9]. According to the World Health Organization (WHO), there were around four million neonatal deaths every year, about a quarter of them is due to neonatal sepsis/pneumonia alone [10].

In Sudan, the prevalence of neonatal sepsis is 17.5% and the mortality is 14.5% [10]. Only few information are available about the bacteria which harbor the vagina of pregnant women and may subsequently cause neonatal meningitis. In addition screening of pregnant for the presence of such bacteria by taking vaginal anal swab is rare in Sudanese hospitals rather than detection of this bacterial antibiogram pattern. Therefore this study was aimed to determine the frequency of ESBL producing *E. coli* and *K. pneuomniae* which isolated from a vaginal swab of pregnant women in Khartoum state.

Material and Method

Patients description

This study included 300 vaginal swabs which were collected from pregnant women in the third trimester (aged 18-43 years) from different gynae Clinic in Khartoum hospitals including Alday-ate Hospital, Omdurman Teaching hospital, and Military hospital. Some of the 129 (43%) clinically diagnosed as having symptomatic genital tract infection while the rest 171 (57%) have no symptoms. The symptoms included vaginal discharge and recurrent urination with dysuria.

Bacteria Isolation and Identification

All swabs were inoculated in the surface of MaCconkey agar media and incubated at 37 °C under the aerobic condition to recover *E. coli* and *K. pneuomniae* from the vaginal swabs. The sample which shows significant growth was identified by using biochemical test including Indole, MR, VP, Citrate and Urease tests in addition to Kligler Iron Agar.

Susceptibility testing

After Identification of Bacteria. A total of 37 (28 *E. coli* and 9 *K. pneuimoniae*) isolates were subjected to Disk-diffusion sensitivity tests were carried out with antibiotic containing disks (Bioanalyse) on Mueller-Hinton agar plate (Himedia). The results were expressed as susceptible or resistant according to the criteria recommended by the Clinical Laboratory Standards Institute (CLSI) (11). The following antimicrobial agents were tested: gentamicin (CN: 10 µg), ciprofloxacin (CIP: 5 µg), trimethoprim-sulfamethoxazole (SXT: 1.25/23.75 µg), imipenem (IMP: 10 µg), cefotaxime (CTX: 30 µg), ceftazidime (CAZ: 30 µg), and ceftriaxone (CRO: 30 µg), Nitrofurantoin (300 µg).

Phenotypic screening for ESBL

Screening of reduced susceptibility to third generation cephalosporins was carried out using Cefotaxime (CTX) and ceftazidime (CAZ) discs and double-disk synergy (DDS) method was used to confirm the presence of ESBLs as recommended by the Clinical and Laboratory Standards Institute [11]. The double-disk synergy test was carried out by placing a susceptibility disk of amoxicillin-clavulanic acid at the center of the plate, and disks containing ceftazidime and cefotaxime were placed 30 mm (center to center) from the amoxicillin-clavulanic acid disk. After aerobic incubation at 37ºC for 18 hours, a clear extension of the edge of the inhibition zone of cephalosporin towards amoxicillin-clavulanic acid disk (key shape) was interpreted as positive for ESBL production.

Statistical analysis

Statistical analysis was performed by using SPSS version 20 (Statistical Package for the Social Sciences). And the result was presented through various graphics and tabulated modules.

Ethics approval and consent to participate

Before conducting study the proposal of the study was ethically approved by ethical committee of Omdurman Islamic University and Ministry of Health. Then informed consent from each patient and permission from the general managers of hospitals were obtained.

Result

300 vaginal swabs were collected from pregnant women in Khartoum state from gynae Clinic, 129 were developed symptoms of vaginal discharge while the rest didn't display any symptoms (Figure 1). Out of 300 vaginal swabs cultivated only 67 (22.3%)

were showed significant growth while 233 (77.7%) show no growth in the surface of the plate after overnight incubation (Figure 2). The result showed that pregnant women who have symptoms of vaginal discharge show more bacterial growth frequencies 45 isolates than those who have no symptoms only 22 isolates (Figure 3). In addition among all isolated bacteria, *E. coli* was the most predominant by 28 isolates followed by *K. pneumoniae* 9 isolates while other bacteria were 30 isolates (Figure 4).

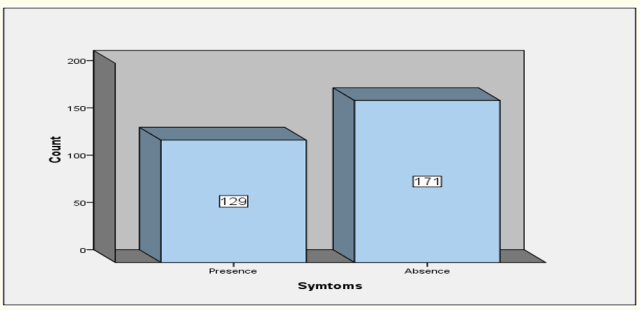

Figure 1: Describe Pregnant women versus symptoms.

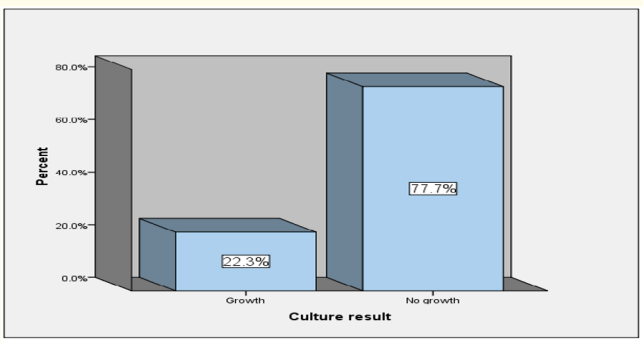

Figure 2: Describe culture result.

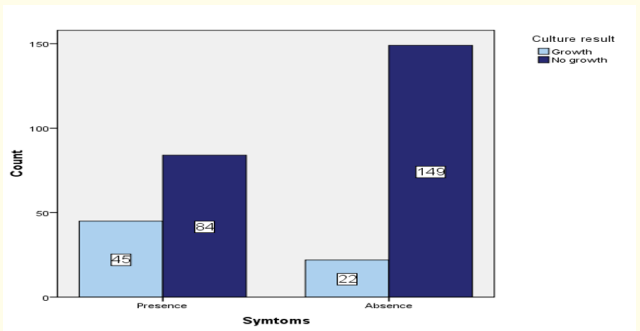

Figure 3: Culture result versus Patients symptoms.

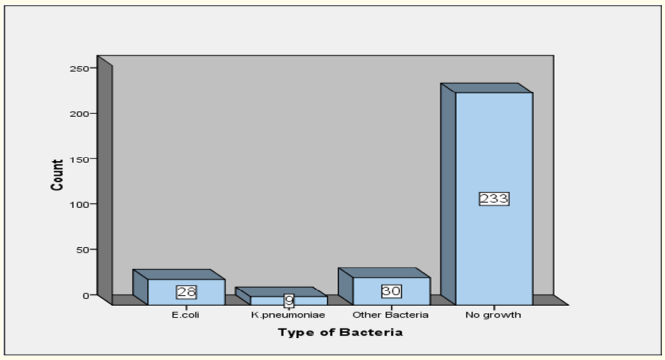

Figure 4: Result of Culture according to the type of Bacteria.

ESBL production has been estimated by the phenotypic method in all *E. coli* and *K. pneuomniae* isolates and the result showed that 7 (25%) out of 28 *E. coli* isolates were produced ESBL. While only 2 (22.2%) K. pneumoniae closest out of 9 were produced ESBL (Figure 5). Among ESBL producing *E. coli* isoles, 71.4% were showed resistant to Cefotaxime and 28.6% were showed a Ceftazidime resistant. In the other hand, all K. pneumoniae isolates which produced ESBL in this study were showed resistant to Cefotaxime (Figure 6). Moreover, the result shows that Ciprofloxacin was most potent antibiotic against ESBL producing *E. coli* and *K. pneuomniae* isolates followed by Imipenem, Gentamycin, Amikacin, Nitrofurantoin, Sulfamethoxazole, and Chloramphenicol by a percentage of 30.4%, 19.6%, 17.4%, 13.0%, 8.7%, 6.5%, 4.3% respectively (Figure 7).

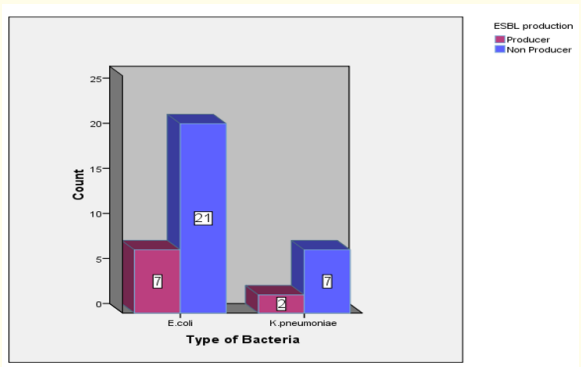

Figure 5: Result of ESBL phenotypic test versus Bacterial type.

Discussion

This study was regarded as the first of its type in our country. Maternal vaginal colonization with *E. coli* and *K. pneuomniae* is a risk for neonatal meningitis [12]. The presences of resistant strains

in the vagina of pregnant women can act as a reservoir for infections in the mother as well as the neonate and interrupting antimicrobial options of them [13].

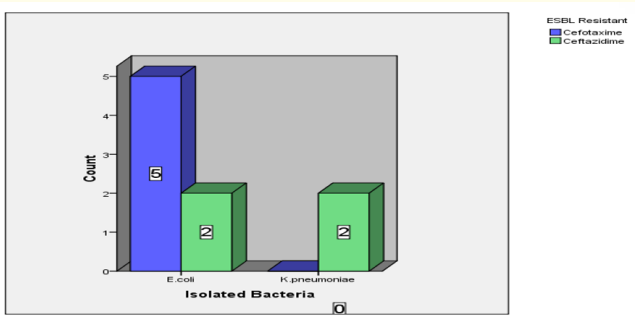

Figure 6: ESBL resistance type versus Bacterial isolates.

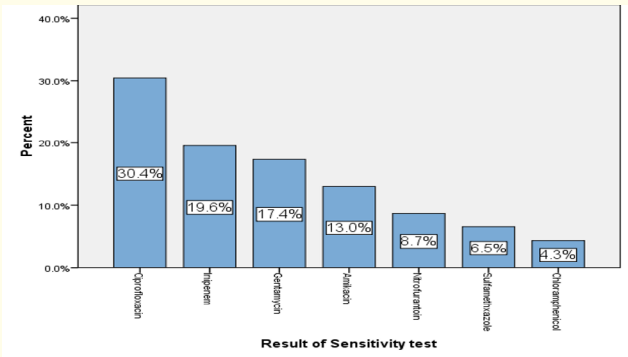

Figure 7: Result of sensitivity test for ESBL producing bacteria.

In our study, our target isolates were *E. coli* and *K. pneuomniae* so we make culture in Macconkery agar under the aerobic condition and our result revealed that *E. coli* was superior to *K. pneuomniae* in vaginal colonization while other bacteria including gram-negative and non-fastidious gram-positive cocci collectively were more than 50% of the isolates.

ESBL was much more produced by *E. coli* isolates than *K. pneuomniae* isolates and the bacteria were showed a high level of Cefotaxime resistant than Ceftazidime with the percentage of 71% and 100% for *E. coli* and *K. pneuomniae* respectively. The cephalosporin resistance observed may be a result of extensive use of this drug as observed by others [13]. Total resistance to Cefotaxime among vaginal *E. coli* isolates in a study in India had shown to be 60% and this result was slightly lower than that obtained by our study [14]. In Argentina, the resistance rate to cefotaxime was found to be 0.8% which is the very low frequency in comparison to our result [14].

In our country drug policies, third generation Cephalosporin including Cefotaxime in addition to Gentamycin is used medically to treat the different types of meningitis especially when Gram-negative infections are suspected [15]. Thus the transmission of these resistant strains to the neonates when occur it will be very serious for neonate and can lead to sepsis and meningitis which may not be able to treated empirically and need culture and sensitivity test which may take some time and the neonate will be under high mortality risk.

Conclusion

This study was done to provide the clinicians with data on the most common organism and their susceptibility pattern in their settings could help them in their practice for prescribing sensitivity specific treatment thus reducing emergence of resistance we conclude that there is a moderate to low prevalence of ESBL producing *E. coli* and *K. pneuomniae* isolates that colonize the genital tract of Sudanese pregnant women and this mount a high risk for neonatal meningitis which could be difficult to be treated. All pregnant women should be screened for the presence of the above-mentioned organism and ESBL screening is highly recommended. Further studies to find out the risk factors associated with carriage of these resistant.

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