Research Article

EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING ENTEROBACTERIACEAE IN WELL-WATER IN ORU

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ABSTRACT: Beta-lactam antibiotics are the largest and most commonly used group of antimicrobial agents world-wide. They show very good tolerability and many of the drugs can be administrated orally. Bacteria expressing extended-spectrum beta-lactamases (ESBLs), enzymes hydrolysingpenicillins and cephalosporins, may not respond to therapy using some of these antibiotics. The isolates are also often co-resistant to other antimicrobial agents, thus further limiting treatment options. Of the 36 isolates collected from well water in OruIjebu, Ogun State and screened using Gram's staining technique, 7 (19.44%) were Gram positive while 29(80.56%) were Gram negative. Out of the Gram's negatives, 23 were identified as members of the enterobacteriaceae family Of the 23 enterobacterial isolates subjected to ESBL detection using DDST, one was found to be positive which is Vibrocholera.. In conclusion this research work shows that the prevalence of ESBL producing Enterobacteriaceae in well water in OruIjebu, Ogun state was very low. Antibiotics sensitivity test reveals that ciprfloxazine, ceptaxidime, cefuroxime, gentamycin, cefotaxime, ofloxacime, nitrofuratoin shows inhibition to Vibrocholera, while there are inhibition to other enterobacteriaceae at varying degree. All isolates show resistance to augmentin while all are susceptible to gentamycin. A majority of the isolates were multiresistant

KEYWORDS: microoranism, enterobacteriaceae, oru, microbs, beta-Lactam

INTRODUCTION

Extended spectrum -lactamases (ESBLs) are enzymes that confer variable level of resistance to oxyiminocephalosporins such as cephotaxime, ceftazidime and monobactams. They occur predorminantly in the family enterobacteriaceae with Klebsiellapneumoniae been the most commonlyreported worldwide and it is responsible for 5-20% of outbreaks of nosocomial infections in intensivecare units, burn, oncology and neonatal units (Kotraet al., 2002).

At present there exist more than 200 different natural variants worldwide which constitute seriousthreat to current -lactam therapies and represent major therapeutic challenges for clinicians (Lin et al.,2005).

Antibiotic resistance in Gram-negative rods is an increasing problem worldwide. Since the introduction of broad-spectrum cephalosporins into clinical practice in the early 1980s, the selective pressure of the use and overuse of antibiotics has resulted in the emergence and rapid development of resistance to expanded spectrum beta-lactam antibiotics (Paterson and Bonomo, 2005; Bradford 2001). Numerous outbreaks of infection with organisms producing extended-spectrum beta-lactamases (ESBLs) have been observed in many countries throughout the world (Bradford, 2001, Paterson et al., 2001), and these organisms have achieved notoriety for causing nosocomial infections that lead to prolonged hospital stay, increased morbidity and mortality, and consequently increased health-care associated costs (Lee et al., 2006; Wollheimet al., 2011).

Since the description of the first ESBL from Germany in 1983 (Knotheet al., 1983), a steady increase in resistance against cephalosporins has been observed (Bradford 2001; Knotheet al., 1983). ESBLs evolved via point mutations of key amino acids in parent, broad spectrum beta-lactamases (TEM-1, TEM-2 and SHV-1). They have an extended spectrum profile that permits hydrolysis of oxyiminocephalosporins and monobactams but not 7-alphamethoxy- cephalosporins (cephamycins). They are generally inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam (Paterson and Bonomo, 2005; Bradford, 2001).

Objectives of Study

- To detect the presence or otherwise of ESBL-producing enterobacteriaceae in well water.
- To discuss transmission
- To provide screening methods
- To identify infection control measures

LITERATURE REVIEW

One of the currently most important resistance mechanisms in Enterobacteriaceae, which reduces the efficacy of modern expanded-spectrum cephalosporins is based on the plasmid mediated production of enzymes that inactivate these compounds by hydrolyzing their b- lactam ring. Such resistance is caused by an increasing number of different point-mutational variants of classical broad-spectrum b-lactamases (BSBL), the so-called extended spectrum b-lactamases (ESBLs). Many ESBLs are members of TEM and SHV b-lactamase families, whereas other groups, such as CTX-M, OXA, and PER b-lactamases have been described more recently (Coqueet al., 2008, Bush et al., 2005). Since the first description of ESBL-producing Enterobacteriaceaefrom hospitalized humans, many nosocomial outbreaks and later also community-associated infections have been reported worldwide (Paterson et al., 2005). Recently, ESBL-producing strains have also emerged in healthy human carriers (Geseret al., 2012), in healthy food-producing animals (Cortés et al., 2010,Geseret al., 2012) and household pets (Ewers et al., 2011) as well as on food products like meat, fish and raw milk (Geseret al., 2012).

Carbapenemases are a diverse group of ß-lactamases belonging to the Ambler classes A, B and D or Bush groups 2f, 3 and 2d, accordingly (Bush et al., 2005) which even inactivate carbapenems. Class A carbapenemases (Bush group 2f) include the serine ß-lactamases NmcA, Sme, IMI-1 and SFC-1 which are chromosomally encoded, as well as the clinically common plasmid encoded KPC enzymes. Carbapenemases of this class are inhibited by clavulanic acid. Class B carbapenemases (Bush group 3) comprise the integron-encoded VIM-types, the IMP-, GIM-1, SPM-1- and SIM-types of enzymes, and the plasmid encoded NDM-1 carbapenamase. These metallo-ß-lactamases are inhibited by EDTA but not by clavulanic acid. Class D (Bush group 2d) consists of OXA-48 type carbapenemases, which are plasmid encoded, and not inhibited by EDTA and not or only weakly inhibited by clavulanic acid. In the last five years, Carbapenemase-producing Enterobacteriaceaehave been increasingly reported in humans world-wide (Nordmannet al., 2011). Moreover, recent data prove that also pigs (Fischer et al., 2012) and cattle (Poirelet al., 2012) constitute in some countries a possible reservoir of carbapenemase producers.

A worrisome aspect is the spread of ESBL- and carbapenemase producers into the environment especially water bodies. Rivers are considered to be of special importance as a reservoir of resistance genes since they are recipients

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of bacteria from different sources. ESBLs undergo continuous mutations, causing the development of new enzymes showing expanded substrate profiles(Ewers et al., 2011).

Biological Characteristics of Extended-Spectrum b-Lactamases (ESBLs)

A recent extensive review discussing themolecular characterisation of ESBLs has been published by Bradford.(Bradford, 2001) Currently, b-lactamases are defined through the classification scheme proposed by Bush and colleagues that is based on molecular characteristics of the gene and enzyme rather than phenotypic hydrolysis characteristics alone.(Bush et al., 1995) ESBLs, which have been isolated from a wide variety of Enterobacteriaceae, (Bradford, 2001) as well as Pseudomonas aeruginosa (Mugnieret al., 1996; Naas et al., 1997) and Capnocytophagaochracea,(Rosenauet al., 2000) are strictly defined as b-lactamases capable of hydrolyzing penicillins, broad- and extended-spectrum cephalosporins, and monobactams, and are inhibited by clavulanic acid (functional group 2be as defined by Bush-Jacoby-Medeiros). These phenotypic characteristics differentiate ESBLs from AmpC type b-lactamases, which are another group of enzymes that are commonly isolated from extended-spectrum cephalosporin-resistant Gramnegative bacteria. AmpC b-lactamases are typically encoded on the chromosome of many Gramnegative bacteria including Escherichia coli, Citrobacterfreundiiand Enterobacterspp., but can also be found on plasmids. (Philipponet al., 2002) AmpC b- lactamases, in contrast to ESBLs, hydrolysebroadand extended-spectrum cephalosporins but are not inhibited by clavulanic acid or other b-lactamase inhibitors.

TEM-Type ESBLs

The native TEM-1 b-lactamase confers resistance to ampicillin, penicillin and first-generation cephalosporins such as cephalothin. This enzyme, which is responsible for 90% of ampicillin-resistance in E. coli isolates, (Livermore, 1995) is also responsible for penicillin resistance in H. influenzaeand Neisseriagonorrhoeae. Mutations within the blatem-1 structuralgene, presumably through antibacterial selection,have allowed the enzyme to expand thehydrolysis capabilities to particular extendedspectrum cephalosporins and aztreonam, while maintaining its original hydrolysis capabilities. TEM-2, the first variant described, differed from TEM-1 through the substitution of a lysine for a glutamine at position 39. However, TEM-2 is not considered an ESBL as the substrate profile is identical to TEM-1. Consequently, amino acid substitutions at 12 separate amino acid positions, acting alone or in concert with other structural gene mutations, have been defined in over 90 described TEM-1-or TEM-2-derived ESBLs.As expected, each TEM-derived ESBL has a slightly different substrate profile in which one ESBL may hydrolyse a specific extended-spectrum cephalosporin more efficiently than another ESBL. Although many ESBLs have subtle differences in substrate profile, these differences cannot be relied upon to differentiate between enzymes and discrimination requires analysis of the amino acid sequence.

SHV-Type ESBLs

The native SHV-1 b-lactamase, found primarily in K. pneumoniae,is a plasmid or chromosomally encoded-enzyme that confers resistance to penicillinsand first-generation cephalosporins. As with TEM-1, specific mutations within the blashv-1structural gene expand the hydrolysis capabilities of SHV-1 to extended-spectrum cephalosporinsand monobactams. Fewer ESBL variants have been described for SHV-1 than with TEM-1.

Other ESBLs

Other ESBLs have recently been described that are not closely related to TEM-1- or SHV-1-derived enzymes.(Bradford, 2001). These b-lactamases, which are found in a variety of different species within the family Enterobacteriaceae and P. aeruginosa, include OXAtype,(Bradford, 2001) CTX-M-type and PER-type (Bradford, 2001) b- lactamases among others. The preferred substrate of these ESBLs differs significantly ranging from cefotaxime (CTX-M-type) to ceftazidime (PERtype).

Although not strictly defined as an ESBL, another group of b-lactamases, called the inhibitor resistant b-lactamases, have been isolated with increasing frequency.(Vedelet al., 1992). The inhibitor resistant b- lactamases are mostly TEM-derived, where 19 separate variants have been described. These TEM variants are resistant to the inhibition of clavulanic acid and sulbactam, but not tazobactam, and do not hydrolyse extended-spectrum

BETA-LACTAM ANTIBIOTICS

Beta-lactam antibiotics are the largest and most commonly used group of antimicrobial agents in Sweden as well as world-wide. The group is distinguished by a chemical structure known as the beta-lactam ring (figure 1a). Based on their chemical structure they can be divided in four different groups, depending on the ring structure fused to the beta-lactam ring (figure 1be), but are often divided in the following groups; penicillins, cephalosporins, carbapenems, monobactam and beta-lactamase inhibitors. (Walsh, 2003)

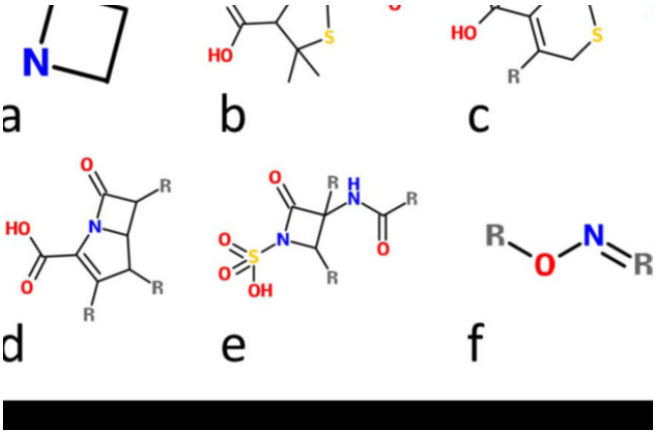

Figure 1) *Chemical structures of a) the beta-lactam ring; the core structure of b) penicillins, c) cephalosporins, d)carbapenems and e) monobactam; and f) an oxyimino group.*

The beta-lactam antibiotics block the transpeptidation of the cell wall component peptidoglycan, through inhibition of penicillin binding proteins, but exactly how this leads to cell death is not known (Walsh , 2003; Andes et al., 2005; Chambers et al., 2005). Since penicillin binding proteins are not found in cells of the Animalia the toxicity of beta-lactams is very low, but allergy against penicillins and other beta-lactams can be very serious (Weiss et al., 2005). Beta-lactam antibiotics are mainly semi-synthetic compounds, originating from fungi and bacteria found in the environment (Walsh, 2003). More than 80 different beta-lactams are in clinical use, but in Sweden only 23 are marketed (Läkemedelsindustriföreningens Service AB. FASS.se, ATC-register, J01, Antibakteriellamedelförsystemisktbruk. [last accessed 9 Feb 2012) Some beta-lactams have a very narrow antimicrobial spectrum, while others have a very broad

spectrum and targets both Gram-positive and Gram-negative bacteria.

Resistance against beta-lactams is primarily mediated by a structural change of the penicillin binding proteins (leading to lower affinity of the drug) or by bacterial production of enzymes cleaving the beta-lactam ring. Other mechanisms are decreased permeability or active transportation via efflux pumps. (Chambers et al., 2005)

Penicillinsare also called penams and are bicyclic (figure 1b). Penicillin G was one of the first antibiotics to be commercialised in the 1940's. They are divided in sub groups depending on their antimicrobial spectrum and stability against penicillinases, and may be administrated orally or parenterally. Penicillins are most often excreted non-metabolised by the kidneys, thus the concentration in urine can reach high levels. (Chambers et al., 2005)

Mecillinam, also known as amidinocillin, is often given as its pro drug pivmecillinam. In Sweden only the oral formula is available (Läkemedelsindustriföreningens Service AB. FASS.se, ATC-register, J01CA08 Pivmecillinam. 2012), and it exerts good activity especially against E. coli, Klebsiellaspp., and Proteus mirabilis. The drug is used in the treatment of uncomplicated UTIs. (The Swedish Reference Group for Antibiotics.Pivmecillinam. 2012)

Temocillinis a narrow-spectrum parenteral drug with very limited availability; it is only approved in the UK and Belgium. It is stable against extended-spectrum beta-lactamases (ESBLs) and can be used in the treatment of septicaemia, UTIs and lower respiratory tract infections caused by Enterobacteriaceae. Clinical use has shown that it can be considered as an alternative in treating infections caused by ESBL producing Enterobacteriaceae, primarily UTIs. (Livermore &Tulkens, 2009)

Cephalosporins, or cephems, are also bicyclic (figure 1c). Although discovered earlier, they did not become commercially available until the 1960's. They all show stability against a wide range of beta-lactamases. (Andes et al., 2005) Noteworthy is the ability of emergence of resistance during treatment of Enterobacterspp., Citrobacterspp., Serratiaspp., Morganellaspp., and Providenciaspp. This is due to either spontaneous mutation or up-regulation of the production of naturally occurring beta-lactamases. (Jones et al., 1997;Jacoby, 2009).Cephalosporins can beadministrated orally, intramuscularly or intravenously, depending on the drug. They are often given empirically in combination with an aminoglycoside or metronidazole (depending on suspected aetiology) for treatment of serious infections such as severe pneumonia, intraabdominalinfections, septicaemia or in patients with febrile neutropenia, but it has been widely debated if combination therapy is favourable or not. In everyday language the cephalosporins are classified into "generations", but for clarity this should be avoided scientifically.

Ceftibutenis an oral cephalosporin approved for the treatment of UTIs and acute exacerbations of chronic bronchitis (Läkemedelsindustriföreningens Service AB. FASS.se, ATC-register, J01DD14 Ceftibuten. last accessed 9 Feb 2012) but SRGA only promotes its use for treatment of UTIs. The antimicrobial spectrum includes the urinary tract pathogens E. coli, Klebsiellaspp. and Proteus spp. and some pathogens of the respiratory tract. (The Swedish Reference Group for Antibiotics.Ceftibuten.last accessed 9 Feb 2012)

Ceftazidimeshows good activity against several Gram-negative bacteria, including Enterobacteriaceae (especially E. coli, Klebsiellaspp. and Proteus spp.), and Pseudomonasaeruginosa. It is administrated parenterally and mainly used for treatment of nosocomial pneumonia and in patients with cystic fibrosis, and suspected septicaemia in neutropenic patients.

Cefotaximeis a broad spectrum cephalosporin for parenteral administration, used for the treatment of serious infections in and from internal organs, skin and soft tissues, including meningitis. The antimicrobial spectrum covers a large portion of the Enterobacteriaceae(especially E. coli, Klebsiellaspp., Proteus spp., Salmonella spp. and Shigellaspp.) and several skin and respiratory tract pathogens. (The Swedish Reference Group for Antibiotics. Cefotaxim.last accessed 9 Feb 2012)

Cefepimeis a broad spectrum cephalosporin with higher stability against beta-lactamases than the other approved cephalosporins available in Sweden. Thus its antimicrobial spectrum almost completely covers the Enterobacteriaceae, including the members naturally producing beta-lactamases. It is also effective against P. aeruginosaand several other Gram-negative non-Enterobacteriaceae, and Gram-positive pathogens of the skin and respiratory tract. It is administrated parenterally and the use is mainly treatment of nosocomial pneumonia and suspected septicaemia in neutropenic patients. (The Swedish Reference Group for Antibiotics.Cefepim. 2012)

Carbapenemsare bicyclic compounds (figure 1d) that came into use in the 1980's. They show very good stability against beta-lactamases, including many of the ESBLs. In combination with their unique mechanism of outer membrane permeability this results in a very broad spectrum of activity, including Gram-positive and Gram-negative aerobic and anaerobic bacteria. All carbapenems are administrated parenterally. (Chambers et al., 2005). Imipenemis always given together with an enzyme inhibitor, cilastatin, preventing its rapid degradation by the kidneys to inactive metabolite (Chambers et al., 2005).

The antibacterial spectrum is very broad and includes P. aeruginosa, Acinetobacterspp., most Enterobacteriaceae (not Proteusspp., Providenciaspp. or Morganellaspp.), Enterococcus faecalis, pathogens of the skin and respiratory tract and many anaerobes. The drug is saved for severe infections originating from internal organs and suspected septicaemia in neutropenic patients, and is effective against infections caused by ESBL producing bacteria, but not against methicillin-resistant staphylococci, Enterococcus faeciumor Stenotrophomonasspp. (The Swedish Reference Group for Antibiotics. Imipenem. 2012)

Meropenemhas an antibacterial spectrum similar to that of imipenem, also including Neisseria meningitides, Proteus spp., and Morganellaspp. This broadens the use, compared to imipenem, to also include meningitis. The activity against enterococci and Stenotrophomonasspp. is insufficient. (The Swedish Reference Group for Antibiotics.Meropenem. 2012)

Ertapenemis a newer carbapenem, with a narrower antibacterial spectrum than the others. It is active against common pathogens of the skin and respiratory tract, Enterobacteriaceae and some anaerobes, but not P. aeruginosaor Acinetobacterspp.(The Swedish Reference Group for Antibiotics. Ertapenem. 2012). The drug is approved for the treatment of pneumonia, intra-abdominal, acute gynaecological and diabetic foot infections (Läkemedelsindustriföreningens Service AB. FASS.se, ATC-register, J01DH03Ertapenem. 2012) but the recommendation of SRGA is to save the drug for treatment of cephalosporin resistant Enterobacteriaceae in the polyclinic setting, since it only requires once daily dosing (The Swedish Reference Group for Antibiotics. Ertapenem. 2012).

Monobactamsare monocyclic compounds (figure 1e), and aztreonamis the only clinically available drug of this group (Chambers et al., 2005).

The antibacterial spectrum is narrow; it covers aerobic Gram-negative bacteria, and the drug is administrated parenterally. Approved indications include gonorrhoea and complicated infections originating from the urinary or respiratory tract. Aztreonam seldom give rise to adverse side effects. (Läkemedelsindustriföreningens Service AB. FASS.se, ATC-register, J01DF01Aztreonam. 2012)

Beta-lactamase inhibitors show very weak antibacterial activity and are used in combinations with penicillins to act as "suicide substrates". They form stable intermediates, thus capturing and deactivating the beta-lactamases. They are stable against penicillinases, some chromosomal cephalosporinases and some ESBLs. (Chambers et al., 2005)

Amoxicillin-clavulanic acid is a combination whose antibacterial activity covers both Gram-negative and Gram-positive, aerobes and anaerobes, found on various sites such as skin, respiratory tract and saliva. It also exerts effect on parts of the Enterobacteriaceae. The drug is used in the treatment of uncomplicated pneumonia and upper respiratory tract infections, UTIs, and bite wounds The Swedish Reference Group for Antibiotics. (Ampicillin/Amoxicillin/Amoxicillinklavulansyra. 2012)

Piperacillin-tazobactamis a parenteral, broad spectrum drug combination used in serious infections such as nosocomial pneumonia, intra-abdominal infections or in patients with febrile neutropenia. The antibacterial spectrum covers pathogens of the skin and respiratory tract, Enterobacteriaceae, Pseudomonas spp. and many anaerobes. (The Swedish Reference Group for Antibiotics.Piperacillin-tazobactam. 2012)

SAMPLE COLLECTION AND ANALYSIS

A total of eighteen samples were collected in OruIjebu, Ogun State in three batches of six each. After which the water was taken to the laboratory for microbial analysis.

Serial dilution was done on the water sample and the 10^5 were used for culturing on MacConkey.

Bacteria were identified by three arrays.

The first one – macroscopic estimation (description of colony).

The second one – microscopic estimation (dyeing by Gram and Schaeffer-Fulton method).

The third one – biochemical tests according to bacteria classification according to Bergey (Holt et al., 1994).

STERILIZATION OF MATERIALS USED

Glassware such as test tubes, pipettes, conical flask, beakers, universal bottles and Bijor bottles were sterilized by washing them with detergents and placed in an oven at 160oC for 3 hours. Foil paper was used to cover opened glassware before they are sterilized. Inoculating loop and needles were sterilized by flaming until red hot and allowed to cool.

GRAM STAINING

The isolates were Gram stained to determine their gram's reaction

i. A colony from the supposed pure culture to be identified was emulsify in physiological saline water on a grease free slide and allowed to air dry.

ii. The dried smear was fixed by passing the slide through a Bunsen flaming for 3 minutes

iii. The dried smear was covered with crystal violet stain for 60 seconds

iv. The stain was rapidly washed off with clean water.

v. The water was tipped off and the smear was covered lugor iodine for another 60 seconds.

vi. The iodine was washed off with clean water.

vii. The smear was rapidly decolourized with ethanol and immediately washed off with clean water.

viii. The smear was then covered with safranin for 60 seconds

ix. The stain was washed off with clear water and left to air dry on a draining rack.

x. The smear was then examined microscopically under the oil-immersion of the microscope.

BIOCHEMICAL TEST

CATALASE TEST

Principle: This test is used to differentiate the bacteria that produces the enzyme catalase, from non catalase producing bacteria.

Procedure:

A 24 hour old culture was used from nutrient agar and tested for catalase production by bringing it into contact with 3% Hydrogen perioxide on a clean glass slide using a sterile wooden stick.

CITRASE TEST

Citrate method using Simmon's citrate agar

1. Aslopes of the medium is prepared in bijou bottles as recommended by the manufacturer (store at 2–8 C).

2. Using a sterile straight wire, a streak on the slope with a saline suspension of the test organism and then the butt is stabbed.

3. And the inoculated bottle is incubate at 35 C for 48 hours. A bright blue colour in the medium indicates positive and retention of the green colour indicate negative result.

OXIDASE TEST

Method:

The edge of microscopic slide was used to pick a colony of the test organisms and placed on a filter paper soaked in 2 to 3 drops of 1 tetremethy1-P phenylenediaminedihydrochloride solutions. A positive reaction is shown by the development of a dark purple colour within 10 seconds, and if there is no dark purplecolouration within 10 seconds.

COAGULASE TEST

O.5ml of undiluted plasma was added to about 2m/s of peptone water in a bijou bottle that was emulsified with the colony of test organisms, incubated at 37°C for 24hours. A positive result is indicated with definite clot formation. A negative result indicates number definite a lot of formation.

INDOLE TEST

The test organisms was inoculated into sterile tryptone soy broth and incubated at 37°C for 48 hours. A drop of Kovac's reagent (4-para-dimethy laminobenzaldehyde) was added and the shock gently and observed for colour change at the interphase of the two fluids. A positive result was indicated by the reformation of a pink to red colour. No colour change from pink to red indicates negatives result.

BLOOD AGAR PLATES (BAP)

This is a differential medium. It is a rich, complex medium that contains 5% sheep red blood cells. BAP tests the ability of an organism to produce hemolysins, enzymes that damage/lyse red blood cells (erythrocytes).

- Beta-hemolysis is complete hemolysis. It is characterized by a clear (transparent) zone surrounding the colonies.

- Partial hemolysis is termed alpha-hemolysis. Colonies typically are surrounded by a green, opaque zone.

If no hemolysis occurs, this is termed gamma-hemolysis. There are no notable zones around the colonies.

ANTIMICROBIAL SUSCEPTIBILITY TESTS

Antimicrobial susceptibility tests were performed as recommended by the Clinical and Laboratory Standards Guidelines (CLSI, 2006) on Mueller Hinton agar plates.

The isolates were cultured on prepared Mueller Hinton Agar (Biotech, England) plates and incubated for at 37oC for 24 hours so as to obtain confluent growth for sensitivity test. Few colonies of isolates from Mueller Hinton plates were dispensed in peptone water and incubated for six hours for sensitivity tests as described by NCCLS (1999).

TEST FOR ESBLS SCREENING

The sensitivity of standard inocula of isolates to Cephotaxime (Ce 30µg), Ceftadime (Ca 30µg) and Ceftazidime/Clavulanic acid (Cac 30µg) (Hi-Media, India) disks was determined on Mueller Hinton Agar (Biotech, India) using Kirby Bueur method (NCCLS, 2002).

RESULTS

Of the 36 isolates collected from the two sampling sites (i.e. well water in OruIjebu, Ogun State) and screened using Gram's staining technique, 7(19.44%) were Gram positive while 29(80.56%) were Gram negative Out of the Gram's negatives, 23 were identified as members of the enterobacteriaceae family

DISCUSSION AND CONCLUSION

The sites of isolation of the test organisms wasOruIjebu, Ogun State. Of the 361 isolates collected from the two sampling sites [i.e. well water in OruIjebu, ogun state] and screened using Gram's staining technique, 7(19.44%) were Gram positive while 29(80.56%) were Gram negative. Out of the Gram's negatives, 23 were identified as members of the enterobacteriaceae family.

On comparing the occurrence and distribution of enterobacterial isolates with E. coli, Klebsiella, Salmonella, Proteus,Enterobacter, and Shigellaspecies being identified among samples obtained from well water in OruIjebu, amongst which Escherichia colihas the highest prevalence (16.67%).

The high occurrence of enterobacteriaceae among the clinical isolates may be due to poor hygienic practices which may result in some of the ESBLs non-producing isolates acquiring plasmids responsible for ESBLs production since plasmids can easily be transferred between organisms living in the same environment and replicate alongside the bacterial chromosome during

reproductive processes (Gunseren et al, 1999).

Of the 23 enterobacterial isolates subjected to ESBL detection using NCCLS breakpoint, one (1) was found to be positive. i.evibrocholeraepositively confirmed to be ESBL producers based on DDST. In general, the percentage prevalence of ESBLs producers among the different species of enterobacterial isolates screened was seen only in Vibro cholera (4.3%). The low occurrence of ESBLs Esherichiaspecies observed in this research is not of less concern since infections caused by these organisms though are very common in this part of the country due to the contagious nature and unavailability of qualitative drinking water respectively yet the occurrency is minimal.

RECOMMENDATIONS

In view of the worldwide occurrence and quick spread of ESBLs among bacterial pathogens and the problems that may be caused by treatment failure due to infections with ESBLs producing organisms, it could be recommended that;

Government should strengthen awareness campaigns on improved hygienic practices so as to reduce the rate of microbial infections as well as spread of ESBLs among both enterobacterial and other bacterial pathogens.

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