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Antimicrobial Susceptibility of Gram Negative Bacteria Species in the Urine of Patients Attending Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA), Ebonyi State, Nigeria.

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ABSTRACT: *The antimicrobial susceptibility of gram negative bacteria species in the urine sample of patients at Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA), Ebonyi State, Nigeria was determined. The antimicrobial analysis was done using standard methods. The result of antibiotic susceptibility test revealed that Salmonella species was highly resistance to amoxycillin- clavulanic acid, bacitracin, ceftriaxone, cloxacillin recording 100% against the test isolate. Escherichia coli isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA) showed 72.2% susceptibility against gentamicin, ceftazidime 80.6%, cefepime and imipenem recording 100%. In vitro antibiotic susceptibility pattern of Pseudonomas aeruginosa , shows low susceptibility data(s) of 5.6, 11.1, 16.7 and 27.8% recorded against mupirocin, ceftriaxone, trimethoprim-sulfamethoxazole and Amoxycillin-clavulanic acid respectively. The result of antibiotic susceptibility test of Klebsiella pneumoniae, Salmonella species, Escherichia coli, Shigella species and Pseudonomas aeruginosa isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA) revealed multiple antibiotic resistance index (MARI) of 0.4, 0.3, 0.3, 0.4 and 0.3 against the test gram negative bacteria.*

Keywords: *Antimicrobial, susceptibility, gram negative, bacteria and Nigeria.*

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I. INTRODUCTION

Gram negative bacteria are bacteria that do not retain crystal violet dye in the gram staining protocol. They are differentiated by their cell wall structure. Some examples of Gram negative bacteria include; Escherichia coli, Salmonella species, Pseudomonas species, Klebsiella species, Proteus species, Helicobacter species, Mosoxella species, Cyanobacteria species, and Spirochetes species. They also constitute a serious problem in urinary tract infections in many parts of the world [1]. Appropriate antimicrobial treatments are often critical to decreasing morbidity and mortality among hospitalized patients having the infections caused by the pathogens. Gram negative bacteria are non-spore forming bacilli that grow rapidly on ordinary laboratory media under both aerobic and anaerobic conditions. It has been estimated that symptomatic urinary tract infections (UTI) occurs in as many as seven (7) million visits to emergency units and 100,000 hospitalized annually [2]. Urinary tract infections (UTI) has been the most common hospital acquired infections, accounting for as many as 35% of nosocomial infection. It is the second most common cause of bacteraemia in hospitalized patients [3]. Urinary tract infections (UTI) is known to occur in all populations but has a particular impact on females of all ages and males at two extremes of life, immuno-compromised patients and anyone with function or structural abnormalities of the urinary and excretory system.

Urinary tract infections (UTI) are known to be the microbial invasion of any of the tissues of the urinary tract reaching from the renal cortex to the urethrameatus [4]. It is also known to be the presence in two consecutive urine samples of greater than 100rods (10^5) organisms per ml of a single bacterial strain in the urinary tract. Urinary tract infections (UTI) can be categorized in ascending or descending order. Infections which are confined to the urethral or the bladder are ascending and referred to as uretitis or cystitis respectively. On the other hand, the pathogens spread from one or other infected body site to the kidney down along the ureter to the bladder is

descending. Such descending urinary tract infections (UTI) cause severe kidney infection, a condition called pyelonephritis [5]. This is potentially more serious; infections to the urethra are called urethritis and to the prostrate gland are called prostitis. This classification is in the presence or absence of symptoms, reoccurrence or absence or presence of complicating factors which are host factors facilitating establishment and maintenance of bacteraemia or worsening the prognosis of urinary tract infections engaging the kidney.

Majority of pathogens are Gram negative species with predominance of members of Enterobacteriace [6]. Escherichia coli accounts for majority of urinary tract infections in young women but other Gram negative rods of different genera such as proteus species and Pseudomonas aeruginosa an aerobic Gram negative rod is also troublesome as a urinary tract pathogens because of its resistance to antimicrobial medicine making it difficult to be treated successfully [7].

Statement of Problem

The incidence of bacterial infection has increased significantly, so contributing to high morbidity and mortality if not treated. This is caused by an increase in antimicrobial resistance and the restricted number of anti-bacterial drugs, which retain many side effects. The most commonorganism responsible for urinary tract infection is the Escherichia coliwhich is a bacterial specie causing up to 80-85% of all symptomatic urinary tract Infection.

Aim of the Study

The study is aimed at assessing the antimicrobial susceptibility of Gram negative bacteria species in the urine of patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA), Ebonyi State.

Objectives of the Study

- To determine the load of Gram negative bacterial species in the urine samples of patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA).
- To isolate and identify the various Gram negative bacterial species associated with the urine samples.
- To determine the prevalence of Gram negative bacteria species in the urine samples of patients attending Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA), Ebonyi State.
- To correlate the age and sex distribution of the patients with respect to the prevalence rate of the isolatedGramnegative bacterial species.
- To determine the antimicrobial susceptibility of the isolated Gram negative bacteria species.
- To determine the MultipleAntibiotics Resistant Index (MARI) of the Gram negative bacterial species identified.

Justification for the study

Gram negative bacteria species are seen as the common cause of urinary tract infection. Appropriate antimicrobial treatment is often critical to decreasing morbidity and mortality among patients having the infections caused by the pathogens. Resistance to antimicrobial drugs makes it difficult to treat the infections successfully [8]. The prevalence of antimicrobial resistance among urinary tract infectious agents is also increasing [9]: [10] and its treatment has become more complicated due to increasing resistance and empirical therapy leading to treatment failures of most associated with Gram negative bacteria [11]. This study will be used to investigate the pattern of Gram negative uropathogens and their antimicrobial resistance pattern among the clinical isolates to the commercially available antibiotics that are often prescribed in urinary tract infectious cases.

II. MATERIALS AND METHODS

Equipments and Instruments used

The various equipments and instruments used for this research include:- Centrifuge (Techmel and Techmel USA), Electron Microscope (B-Bran, UK), Autoclave, incubator (Techmel and Techmel, USA), Hot air oven (Gallencomp/ England China), Water bath (Thermostatic, China), Refrigerator (Haier ThermacoolNigeria), Analytical Balance (Better Toledo PL 303), Handglove(Agary PharmaceuticalLimited), nose mask, sample collection container, masking tape, Bunsen burner, funnel, test tubes, pipette, sterile petri dishes, slides, wire loop, stirrer, beaker, cotton wool, scissors.

Chemical and Reagentsused include:-Oxidase reagent, Crystal violet, peptone, lactose,lugolsiodine solution, indole, alcohol, Saffranin,glucose fermentation,Sodium chloride, kovac's reagent (Fisher Scientific Company USA), Hydrogen peroxide (H_2O_2) solution, distilled water 1 liter.

Media Used:

The media that were used in this study includes; MacConkey Agar, Nutrient Agar, Nutrient Broth, Muller Hinton Agar, (Oxoid, UK), Endor Agar, Blood Agar, Urea Agar, Simon's Citrate Agar.

MEDIA PREPARATION

All Media were prepared following manufacturer's instructions.

MacConkey Agar

A 12.1g of MacConkey agar was measured and dissolved in 500ml of distilled water. Then it was sterilized by autoclaving at 121^0C for 15 minute at 15 psi and allowed to cool to 45^0C before pouring into petri dish soap and allowed to solidify on the bench and incubated at 37^0C for 24hrs to ascertain sterility.

NUTRIENT AGAR (NA)

The nutrient agar used was prepared by weighing and dissolving 14.0g of nutrient agar powder in 500ml of distilledwater in 1000ml conical flask. This was properly mixed and heated over Bunsen flame to dissolve. Then it was sterilized by autoclaving at 121^0C for 15 minute at 15psi and allowed to cool to 45 ^{0}C before pouring 20ml each into petri dishes. They were left on the bench to solidify and incubated at 37^0C for 24hrs to ascertain sterility. [12]. This media is used for the enumeration of bacterial cells and also to maintain pure culture.

NUTRIENT BROTH (NB)

Exactly 6.5g nutrient broth powder was weighed and dissolved in 500ml of distilled water. 5ml each was collected and inoculated into 200 test tubes respectively and corked properly. It was sterilized by autoclaving at121^0C for 15 minutes at 15psi. It was allowed to cool to 40^0C before inoculation of the test sample.

MUELLER-HINTON AGAR

A 19.5g of Mueller Hinton agar was dissolved in 500ml of distilled water in a beaker. This was properly stirred and mixed to homogenize. The medium was sterilized by autoclaving at 121^0C for 15 minutes at 15 psi and allowed to cool at 45^0C before pouring into petri dishes and allowed to solidify on the bench and incubated at 37^0C to ascertain sterility [13].

ENDO AGAR

41.5 grams of Endo agar was suspended in 1000ml of distilled water. Then 4ml of 10% Basic fuchsin (FD059) was added and it was heat to boiling to dissolve the medium completely. It was sterilized by autoclaving at 121^0C for 15 minutes at15psi. It was mixed well before pouring into the sterile petri plates. The specimen was streaked as soon as possible after I received it in the labouratory. The streak plate was used primarily to isolate pure culture from specimens containing mixed Flora. Alternatively, if the material was being cultured directly from a swab, the swab was rolled over a small area of the surface at the edge and then streak from the inoculated area. The Incubated plates were protected from light at 35 ± 2^0C for 18 to 24hrs. This media is a slightly selective and differential medium used for the isolation and differentiation of Enteriobacteriaceae and other Gram negative rods from clinical specimen.

BLOOD AGAR

40 grams of blood agar was dissolved in 950.0ml of distilled water and was mixed thoroughly. It was heated frequent agitation and boil for 1minutes to completely dissolve. The media was sterilized by autoclaving at 121^0C for 25 minutes at 15psi. It was allowed to cool at 45^0Cto 60^0C. Then 50ml of sterile defibrinated sheep blood was aseptically added, mixed thoroughly and pour into sterile petri dishes.Blood Agar is an enriched growth medium that encourages bacterial growth.

UREA AGAR

24.0grams of urea agar was suspended in 950ml of distilled water. It was heated to boiling in order to dissolve the medium completely. The media was sterilized by autoclaving at pressure of 115^0C for 20 minutes at 10psi. It was allowed to cool to 45 to 50^0C and the 59ml of sterile 40% urea solution (FD048) was added aseptically and mixed well. It was dispensed into sterile tubes and were allowed to set in a slanting position. I ensured that the medium was not overheated or reheated because urea decomposes very easily.

Thisculture media is used to detect rapid urease activity of the proteae and non- rapid urease activity of someEnterobacteriaceae.

SIMMONS CITRATE AGAR

It was used in the differentiation of gram negative enteric Bacilli based on citrate utilization.

The Simmons citrate agar salts was dissolve in deionized water and was adjusted to pH 6.9. TheAgar and bromothymolblue was added, gently heated with mixing to boiling until agar is dissolved. The medium was used either as slopes in test tubes or as a plate medium in petri dish soap. In both cases, the surface of the medium was lightly inoculated by streaking and where slopes are used, the butt of medium was innoculation by stabbing. For tubes, 4.0 to 5.0ml was dispense into 16mm tubes. Autoclave was done at 121 ^{0}C under 15 psi pressures for 15 minutes. It was cooled in slanted positioneither long slant or shallow butt. The tubes was stored in a refrigerator to ensure a shelf life of 6 to 8 weeks. The uninoculated medium was a deep forest green due to the pH of the sample and the bromothymol blue. The positive growth (i.e citrate utilisation) produces an alkaline reaction and changes the colour of the medium from Green to brighter blue example Klebsiella, Proteus, Enterobacter species etc.Negative test (i.e no citrate utilisation) the colour of the medium remains unchanged example Escherichia coli, Shigella, Yersina, Edwardsiellaspecies etc.

STUDY AREA

The study was carried out at Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA). Alex Ekwueme Federal Teaching Hospital Abakaliki (AE-FETHA) is in Abakaliki town, the capital city of Ebonyi State, Nigeria. Abakaliki the capital of the present day Ebonyi State is in Southeastern Nigeria. Its geographical coordinates lie within Longitude 8.06'E and Latitude 6.20'N. It is situated at an elevation of 117 meters above sea level. Abakaliki is populated and inhabited by indigenes and people from other parts of Nigeria. Ebonyi State shares a border with Benue State to the North, Enugu State to the West, Imo and Abia to the South and Cross River to the East. The tropical climate is broadly of two seasons which are the rainy season between April and November and dry season between December and March. Temperature throughout the year ranges between 21.7^{0}C to 24.7^{0}C. Humidity ranges between 50% - 70% during the rainy season. The annual rainfall is about 1-150mm. It's vegetation is if the sub-savannah rain forest. The inhabitants are primarily members of the Igbo nation. It used to be the head quarters of the old Abakaliki zone in the old Anambra and Enugu State before the creation of Ebonyi State in 1996. Abakaliki is made up of four clans namely Ezza Ezekuna, Ngbo, Izzi and Ikwo. It has estimated population of 141,438 according to the 2006 census [14]. Its last known estimated population stand at 438,700 [15].

Figure 1: Map of Ebonyi State, showing Abakaliki the capital of Ebonyi State [16].

ETHICAL CLEARANCE

The approval for this study was gotten from theResearch and EthicsCommittee of Alex Ekwueme Federal Teaching Hospital AbakalikiEbonyi State.

STUDY DESIGN

This study is a prospective, experimentalstudy which involves collection of urine samples and the samplescollected were used to carrying out the study. It also involves the use of questionnairedesigned for collection of the patients data.

SAMPLE SIZE DETERMINATION

Sample size in this study was determined using Cochran's formula. Which is based on the previous studies carried out on the prevalence rate of urinary tract infection (UTI) caused by the Gram negative bacteria species in some other hospitals in Nigeria using urine samples. In order to obtain the sample size, the mean from the previous work was taken as 0.05%. The following is the Cochran's formula.

$n_0 = \frac{Z^2pq}{e^2}$

Where n_0= Confidence level at 95% equivalent to Z = 1.96

Z= Standard normal deviation at 95% confidence interval (which was 1.96).

P = Proportion of the target population gotten from the mean taken from the previous studies (0.5).

q = Alternate proportion (1-P)

e= Desired level of precision (take margin error as 0.05%)

Hence $n_0 = \frac{(1.96)^2 (0.5)(0.5)}{(0.05)^2}$

Minimum sample size = 385

SAMPLE COLLECTION

Prior to the start of the research work, permission and informed consent through a letter was seeked from the chairman, research and ethics committee and from the patients attending Alex Ekwueme Federal Teaching Hospital, (AE-FETHA) Abakaliki. A questionnaire designed for collection of their individual data was completed by the patients. A total of One Hundred and Fifty (150) freshlyvoided mid-stream urine samples was collected from Out-patients department both male and female patients. The samples were collected with a clean, dry, and wide mouth transparent EDTA sample collection container, put into a polythene bag andwere instantly transferred using Staurt transport medium tomicrobiology laboratoryunit of Ebonyi State University Abakaliki, Nigeria immediately for bacteriological analysis [17].

On the urine sample the name, age, sex including the date and time of collection were indicated. Only patients who have not been on antibiotic preceding one week of sample collection and patients not more than 60 (sixty) years of age was enrolled and used for the experiment.Patients that has been on antibiotics less than one week before sample collection, those that are currently on antibiotics and those that have never taken antibiotics before was excluded.15-20ml of clean catch mid-stream urine samples was collected after clear instructions on collections including cleaning the genitalia before voiding of urine.

SAMPLE ANALYSIS

A total of One Hundred and Fifty (150) early morningfreshly voided mid-stream urine samplesof both male and females patients attending Alex Ekwueme Federal Teaching Hospital (AE-FETHA) Abakaliki were collected from the Out-patients department. The samples were collected using clean, dry, and wide mouth transparent EDTA sample collection container. Theurine samples collectedwas observed macroscopically that is, with the naked eyes to ascertain if the urine was clear or cloudy (turbid) and the colour of the urine was also observed.

The urine wasmixed thoroughly by rotating the container clockwise and anticlockwise directions. A loopful of the collected urine sampleswas inoculated aseptically into a sterilized nutrient brothand incubated at 37^0C for 24 hours. A loopful of 24 hours turbid broth culture was each plated by streaking peripherally over the surface of a solidified MacConkey agar and also incubated at 37^0C for 24hrs. All plates were incubated at 37^0C aerobically for 24hrs.

After an overnight incubation of the cultured plates, the plates were examined macroscopically by observing consistency, the colonial appearance of pink colonies of the organisms on the agar plates which was sub-cultured onto the nutrient agar (Oxoid, UK). The axenic culture obtained weresubjected to Gram staining and some biochemical testssuch as Oxidase test, Catalase test, Motility test, Methyl red test, indole test, Urease test,Citrate Utilization Test (Simmons Citrate Agar). These test is aimed at identifying the bacteria isolates and its microscopy was also carried out.

GRAM STAINING

This was carried out using clean grease free slides. A flamed wire loop was used to aseptically pick a colony of each test organisms and was placed on theclean grease free slides and air dried. The smear was allowed to dry and was heat fixed by waving over flame of a Bunsen burner.The smear was then covered with crystal violet reagent for 30-60 seconds. The stained slide was placed on a rack over a sink and rinse in a slowly running tap water. The film was covered with lugol's iodine for30 minutes and was washed off with clean tap water. The smearwas decolourized with acetone-alcoholand immediately washed with clean tap water slowlyuntil nomore dye runs out. The stained slides were counter stain with Saffranin reagent for thirty (30) to sixty(60) seconds and rinsed in slowly running water. The slides were air dried and the under surface blotted before viewing under the microscope. The stained slide was viewed with oil immersion lens x100 of the microscope. Gram positive bacteria appeared purple while Gram-negative cells appeared pink or red.

BIOCHEMICAL TESTS

This was used for the identification of the exact organism present in the urine specimens. The biochemical tests used are:

- Oxidase Test
- Catalase test
- Motility test
- Methyl red test
- Indole test
- Urease test
- Citrate utilisation test.

OXIDASE TEST

A piece of filter was placed in a clean petri dish and 2-3 drops of freshly prepared oxidase reagent was added on it. With the aid of glass rod, a colony of the tested organism was removed and smeared on the filter paper. This was observed for blue-purplecolour appearance after few seconds.

CATALASE TEST

This test was used to demonstrate which of the isolation could produce the enzyme catalase that release oxygen from hydrogen peroxide (H_2O_2). This test is usually used as an acid to differentiate Staphylococci from Streptococci and to differentiate other catalase positive organisms from catalase negative organisms [18].

A drop of 3% Hydrogen peroxide(H_2O_2) solution was placed on a clean, grease-free glass slide; sterile wire loop was used to pick the test organisms and dipped into the Hydrogen peroxide (H_2O_2) solutionand observed immediately for bubble formation which indicated;

Active bubbles – positive catalase test.

No bubbles –negative catalase test.

MOTILITY TEST(Hanging Drop Method)

This test wasused to determine which of the isolates is motile. Motility test is usually used to differentiate motile organisms from non-motile ones. For this test the hanging drop technique was carried out as described by [19].

Two drops of wet suspension of the test organism was placed on a grease-free coverslip and quickly inverted over a depression grease-free slide supported by a ring of petroleum jelly. It was examined under the microscope with x10 and x40 objective lenses. Visible movement of the organism indicate motility, but if absent shows no mortality [20].

METHYL RED TEST

This test was used to detect which of the isolates could produce and maintain sufficiently a stable acid product from glucose fermentation. The test is usuallyused as an acid in the identification and differentiation of the Enterobacteriacae [21]. This test was carried out as described by [22].

The alkali oxidizes the acetyl methyl carbonyl (acetonic) diacetyl which gives the pink colour.

INDOLE TEST

This test was used to differentiate the Enterbacteriacea such as Escherichia coli which is negative. Indole positive bacteria break down the amino acid tryptophan with the release of indole.

A dense suspension of the test organism in 24 hours nutrient broth culture was added with 3ml of Kovac's reagent and observed for red surface layer appearance within 3 minutes which indicates indole positive and yellow surface layer shows negative result.

METHOD

- The test organism wasinoculated into peptone water containing tryptophan.
- The broth was incubates at 35°C for 48hrs.
- Indole was tested by adding 0.5ml kovac's reagent and shak. Indole positive which form a red colour layer when kovac's reagent was added after 10mins.

RESULTS

Red surface layer – positive indole test

No red surface – negative indole test

UREASE TEST

This test was used to demonstrate the ability of isolate to produce the enzyme urease which splits urea forming ammonia. This test is usually used to differentiate organisms like proteus from non-urease positive organisms [23]. The methods used were described by [24].

METHOD

A loop full of the isolate was used to inoculate a tube of urea agar. The tubes were incubated at 37°C. A change in colour from yellow to red confirm the presence of urease.

RESULTS

- Red colour- positive urease test
- No red colour – negative urease test

CITRATE UTILISATION TEST (Simmons Citrate Agar)

This test was used to identify which of the isolates can utilise citrate as the sole source of carbon for metabolism. The test is usually used as an acid in the differentiation of organisms of Enterobacteriacae and most other genera [25]. The medium used for this test was the Simmons citrateagar. The principle was based on the ability of an organism to utilize citrate as the only source of carbon and ammonium as the only source of nitrogen.

METHOD

The Simmons citrate agar was prepared by weighing 9.1g of the agar and dissolved in 250ml of distilled water. A 5ml each of the solution was inoculated into the test tubes and sterilized by autoclaving at 121°C for 15 minutes at 15psi. These test tubes were slanted and allowed to cool to 45°C on the bench. The isolates were inoculated into each of the test tubes and incubated at 37°C for 24hrs. Change in colour from Green to blue colouration indicated positive citrate test [26].

TRIPLE SUGAR IRON AGAR

This media is used to differentiate Enteriobacteria from non-fermentative gram-negative bacteria such as Pseudomonas aeruginosa etc. Exactly 1.7g of Triple Sugar Iron Agar was weighed and dissolved in 100ml if distilled water. A 5ml volume was pipetted into a test tube and sterilized by autoclaving at 121°C for 15minutes at 15psi and allowed to cool to 45°C (placed to firm slant 3cm each) before inoculation of the isolates 3-5mm into the bottom of the tube and incubated at 37°C for 24hrs to ascertain sterility [27].

Preparation of 0.5 Macfarland Turbidity Standards

An equivalence of 0.5 Macfarland Turbidity Standard was prepared by adding 1ml of concentrated sulphuric acid (H_2SO_4) to 99ml of distilled water to give 100ml and 0.5g of dehydrated Barium Chloride ($Bacl_2$-$2H_2O$) and was dissolved in 50ml of distilled water separately. A calibrated pipette was used to add in drop wise of 0.5ml of BariumChloride solution to 99.5ml of the sulphuric acid solution in separate test tube to bring the volume

to 100ml in a volumetric flask with 1%H_2SO_4. This was mixed thoroughly for 3-5minutes while examining virtually, until the solution appeared homogenous and free from clump to obtain 0.5Macfarland Turbidity Standards. Aliquots of the standard solution was transferred to screw capped test tubes and stored at room temperature (28°C). This was used to adjust and compare the turbidity of the test bacteria in order to obtain a confluent growth [28].

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial sensitivity was tested for each isolated organism using the disc diffusion methodof Kirby-Bauer as described by the National Committee for Clinical Laboratory Standard Institute (NCCLI, 2001).

Muller-Hinton agar platewas prepared and standardized innoculum of each isolate was inoculated on each of the Muller Hinton agar plates respectively.An overnight culture of the test bacteria grown in nutrient broth was adjusted to 0.5 Macfarland turbidity standards. 0.5 Macfarland equivalent standard of the test organisms was inoculated on the surface of the Muller-hinton (MH) agar plates usinga sterile wire loop. The test organisms were collected by inserting the flamed wireloop and allowing it to cool then wire vertically into the urine. Theloopful urine was streaked peripherally over the surface of the Macckonkey agar. The following antibiotics discs and their concentration whichare Amoxicillin/Clavulanic acid 30µg, Bactracin (B) 10units, Ceftazidine (CAZ) 30µg, Ceftriaxone (CRO) 30µg, Cefoxitin (FOX) 30µg,Cefepime 30µg, Cloxacillin 5units, Gentamycin(GEN) 30µg,Imipenem (IPM) 10µg,Mupirocin (MUP) 15µg, Sulphamethoxazole /Trimethoprim (SXT) 30µgasimpregnatedasceptically on the plate. The antibiotics were allowed to diffuse for about 10 minutes and the plateswas incubated at 37^0C aerobically for 24hrs. The plates was examined macroscopically. The bacterial colonies was counted and multiplied by 100 to give an estimate of the number of bacteria present per millimetre of urine. A significant bacterial count was taken as any count equal or in excess of 10,000cfu ml. Representative of growing colonies was picked with repeated streaking. Pure cultures obtained was used for biochemical testswhich is aimed at identifying the bacteria isolates and its microscopy was also carried out.

The antimicrobial diffused from the disc to the medium and the growth of the organism was inhibited at a distance from the disc that is associated to the sensitivity of the organism. Strains that was sensitive to the antimicrobial was inhibited at a distance from the disc where as resistant strains had smaller zones of inhibition. The zones of inhibition was measured with meter rule and compared with clinical and labouratory standard institute (CLSI) guidelines [29].

Determination of Multiple Antibiotics Resistance Index

Multiple Antibiotic ResistanceIndex was determined according to the method described by [30] [31] and the following formula was used to determine Multiple Antibiotics Resistance Index (MARI);

MARI = a/b

Where a= Number of antibiotics to which the test isolated showed resistance.

b= Total number of antibiotics to which the test isolate has been evaluated or showed sensitivity.

RESULTS

Gram negative bacteria isolated from urine samples of patients attending Alex Ekwueme Federal University Teaching Hospital Abakaliki were identified morphologically through Grain staining and motality test. Biochemical characterization which include Urease, Voge- Please, Citrate, Indole, Methyl red, Oxidase and Sugar fermentation as explained in Table 1.

Table 1: Morphology and Biochemical characterization of Gramnegativebacteria isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Gram staining	Shape	Colour	TSI	Oxidase	Motility	Urease	VP	Methy red	Indole	Citrate	Glucose	Lactose	Mannitol	Maltose	Sorbitol	Xylose	Suspected Organism
-	Rod	Black	A	-	+	-	-	+	-	-	+	-	+	+	+	+	Salmonella species
-	Rod	Green	A	+	+		-	-	-	+	+	-	+	-	-	+	Pseudomonas aeruginosa
-	Rod	Pink and drew	A	-	-	+	+	-	-	+	+	+	+	+	+	+	Klebsiellapneumonia
-	Rod	Pink	A/G	-	+	-	-	+	+	-	+	+	+	-	+	-	Escherichia coli
-	Rod	Light brown	A	-	-	-	+	-	-	-	+	-	+	-	+	-	Shigellaspecies

KEYS: TSI-Triple Sugar Iron, VP-Voges-Proskauer, (+)- Positive, (-)- Negative, A-Acid, A/G-Acid/Gas

In Table 2, Demographic data of patients with reference to gender reveals a prevalence rate of 67(44.7%) and 60(40%). Gram negative bacteria for both male and female respectively. High prevalence rate of 26% . Gram negative bacteria was documented against age group 21-30years while age group 41-50years recorded the least prevalence rate of 6.7% Gram negative bacteria as presented in Table 2. Gram negative bacteria were more predominant in urine sample of outpatient recording 50.7% while 34% was documented against gram negative bacteria [32].

Table 2: Demographic profile of patients with Gramnegativebacteria with regards to gender, age and hospitalization.

Variable		**Number of specimen**	**Presence of Gram negative bacteria (%)**
Gender	Male	75	67(44.7)
	Female	75	60(40)
	Total	**150**	**127 (84.7)**
Age group	11-20	29	22(14.7)
	21-30	45	39(26)
	31-40	41	38(25.3)
	41-50	14	10(6.7)
	51-60	21	18(12)
	Total	**150**	**127 (84.7)**
Hospitalization	In-patient	75	51(34)
	Out-patient	75	76(50.7)
	Total	**150**	**127 (84.7)**

Table 3 shows thatEscherichia coli was more predominant in urine sample recording 24% while 20%, 18% 12% and 10.7% was documented against Salmonella species, Shigellaspecies, Pseudonomas aeruginosa and Klebsiella Pneumonia respectively as presented in Table 3 [33].

Table 3: Occurrence rate of Gramnegativebacteria isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Gramnegativebacteria	**Occurrence rate (%)**
Klebsiellapneumoniae	16(10.7)
Salmonella species	30(20)
Escherichia coli	36(24)
Shigellaspecies	27(18)
Pseudonomas aeruginosa	18(12)
Total	**127 (84.7)**

Klebsiella pneumoniae isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA) in this study was 6.3, 25, 93.7 and 100% susceptible to gentamicin, ceftazidime, cefepime and imipenem respectively as shown in Table 4 [34].

Table 4: Antibiotic susceptibility test of Klebsiellapneumoniaeisolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Antibiotic	**Disc potency (µg)**	**No. of isolate**	**Resistance (%)**	**Susceptibility (%)**
Amoxycillin-CA	30	16	16(100)	0(0.0)
Bacitracin	10	16	16(100)	0(0.0)
Ceftazidime	30	16	12(75)	4(25)
Ceftriaxone	30	16	16(100)	0(0.0)
Cefoxitin	30	16	16(100)	0(0.0)
Cefepime	30	16	1(6.3)	15(93.7)
Cloxacillin	5	16	16(100)	0(0.0)
Gentamicin	30	16	15(93.7)	1(6.3)
Imipenem	10	16	0(0.0)	16(100)
Mupirocin	15	16	16(100)	0(0.0)
Trimethoprim-sulfamethoxazole	30	16	16(100)	0(0.0)

Key: Clavulanic acid

The result of antibiotic susceptibility test revealed that Salmonella species was highly resistance to amoxycillin- clavulanic acid, bacitracin, ceftriaxone, cloxacillin recording 100% against the test isolate as shown in Table 5 [35].

Table 5: Antibiotic susceptibility test of Salmonella species isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Antibiotic	**Disc potency (µg)**	**No. of isolate**	**Resistance (%)**	**Susceptibility (%)**
Amoxycillin-CA	30	30	30(100)	0(0.0)
Bacitracin	10	30	30(100)	0(0.0)
Ceftazidime	30	30	16(53.3)	14(46.7)
Ceftriaxone	30	30	30(100)	0(0.0)
Cefoxitin	30	30	20(66.7)	10(33.3)
Cefepime	30	30	0(0.0)	30(100)
Cloxacillin	5	30	30(100)	0(0.0)

Gentamicin	30	30	11(36.7)	19(63.3)
Imipenem	10	30	0(0.0)	30(100)
Mupirocin	15	30	29(96.7)	1(3.3)
Trimethoprim-Sulfamethoxazole	30	30	24(80)	6(20)

Key: Clavulanic acid,

As presented in Table 6, Escherichia coli isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA) showed 72.2% susceptibility against gentamicin, ceftazidime 80.6%, cefepime and imipenem recording 100% [36].

Table 6: Antibiotic susceptibility test of Escherichia coli isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Antibiotic	**Disc potency (µg)**	**No. of isolate**	**Resistance (%)**	**Susceptibility (%)**
Amoxycillin-CA	30	36	34(94.4)	2(5.5)
Bacitracin	10	36	36(100)	0(0.0)
Ceftazidime	30	36	7(19.4)	29(80.6)
Ceftriaxone	30	36	36(100)	0(0.0)
Cefoxitin	30	36	36(100)	0(0.0)
Cefepime	30	36	0(0.0)	36(100)
Cloxacillin	5	36	36(100)	0(0.0)
Gentamicin	30	36	10(27.8)	26(72.2)
Imipenem	10	36	0(0.0)	36(100)
Mupirocin	15	36	36(100)	0(0.0)
Trimethoprim-Sulfamethoxazole	30	36	36(100)	0(0.0)

Key: Clavulanic acid,

Shigella specieselucidate least susceptibility data (3.7%) against cephalosporin (ceftriaxone) while high susceptibility data at 55.6, 88.9 and 100% was documented against gentamicin, imipenem and cefepime as comparactively shown in Table 7 [37].

Table 7: Antibiotic susceptibility test of Shigella species isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Antibiotic	**Disc potency (µg)**	**No. of isolate**	**Resistance (%)**	**Susceptibility (%)**
Amoxycillin-CA	30	27	27(100)	0(0.0)
Bacitracin	10	27	27(100)	0(0.0)
Ceftazidime	30	27	27(100)	0(0.0)
Ceftriaxone	30	27	26(96.3)	1(3.7)
Cefoxitin	30	27	27(100)	0(0.0)
Cefepime	30	27	0(0.0)	27(100)
Cloxacillin	5	27	27(100)	0(0.0)
Gentamicin	30	27	12(44.4)	15(55.6)
Imipenem	10	27	3(11.1)	24(88.9)

Mupirocin	15	27	27(100)	0(0.0)
Trimethoprim-Sulfamethoxazole	30	27	27(100)	0(0.0)

Key: Clavulanic acid,

In vitro antibiotic susceptibility pattern of Pseudonomas aeruginosaas arrange in Table 8, shows low susceptibility data(s) of 5.6, 11.1, 16.7 and 27.8% recorded against mupirocin, ceftriaxone, trimethoprim-sulfamethoxazole and Amoxycillin-clavulanic acid respectively [38].

Table 8: Antibiotic susceptibility test of Pseudonomas aeruginosaisolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Antibiotic	**Disc potency (µg)**	**No. of isolate**	**Resistance (%)**	**Susceptibility (%)**
Amoxycillin-CA	30	18	13(72.2)	5(27.8)
Bacitracin	10	18	18(100)	0(0.0)
Ceftazidime	30	18	18(100)	0(0.0)
Ceftriaxone	30	18	16(88.9)	2(11.1)
Cefoxitin	30	18	18(100)	0(0.0)
Cefepime	30	18	0(0.0)	18(100)
Cloxacillin	5	18	18(100)	0(0.0)
Gentamicin	30	18	0(0.0)	18(100)
Imipenem	10	18	0(0.0)	18(100)
Mupirocin	15	18	17(94.4)	1(5.6)
Trimethoprim-Sulfamethoxazole	30	18	15(83.3)	3(16.7)

Key: Clavulanic acid,

The result of antibiotic susceptibility test of Klebsiella pneumoniae, Salmonella species, Escherichia coli, Shigellaspecies andPseudonomas aeruginosaisolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA) revealed Multiple Antibiotic Resistance Index (MARI) of 0.4, 0.3, 0.3, 0.4 and 0.3 documented in Table 9 against the test Gram negative bacteria.

Table 9: Multiple Antibiotic Resistance Index of Gramnegativebacteria isolated from urine sample of patient attending Alex Ekwueme Federal University Teaching Hospital, Abakaliki (AEFEUTHA).

Gramnegativebacteria	**Multiple Antibiotic Resistance Index (MARI)**
Klebsiellapneumonia	0.4
Salmonella species	0.3
Escherichia coli	0.3
Shigellaspecies	0.4
Pseudonomas aeruginosa	0.3

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