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Multidrug resistant profiles of clinical isolates of *Escherichia coli, Salmonella typhi* and *Shigella* species

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Abstract

In order to determine the antibiotic susceptibility pattern and multi antibiotic resistance index of *E.coli, Salmonella typhi and Shigella* species, a total of 32 pre-identified clinical isolates were obtained and subjected to conventional biochemical and gram staining reaction for confirmation. Antibiotic susceptibility testing to ten commonly prescribed antibiotics (Amplicin (PN:30 mcg), Gentamicin (GEN:10 mcg), Streptomycin (STR:30 mcg), Septrin (SXT:30 mcg), Nalidixic acid (NAL:30 mcg), Ciprofloxacin (CIP:10 mcg), Augmentin (AU:30 mcg), Reflacin (PEF:10 mcg), Tarivid (OFX:10mcg), and Ceporexin (CEP:10 mcg) was considered using disc agar diffusion method and reported with the Clinical and Laboratory Standard Institute Interpretative chart. Zones of inhibition were measured and Multiple Antibiotic Resistance index was calculated. *Salmonella* isolates demonstrated a high rate of susceptibility to Tarivid (90%), Reflaxin (90%), Gentamycin (80%), ciprofloxacin (60%), Augmentin (80%) and moderately susceptible to Streptomycin (40%) and Septrin (20%) with high resistance to Nalidixic acid (90%), Amplicin (90%) and Ceporexin (70%). Nearly all *Shigella* isolates were susceptible to Augmentin (80%), Reflaxin (90%), Tarivid (80%), intermediate susceptibility was also noted in Streptomycin (40%) and Ceporexin (50%) while high resistance to ciprofloxacin (80%), Amplicin (90%), Septrin (70%) and Nalidixi acid (90%). High rate of resistance was observed to nearly five antibiotics in *E,coli* with low susceptibility rate to all the antibiotics tested. All the isolates had Multiple Antibiotic Resistance index greater than 0.2 and above. This may be as a result of previous exposure to antibiotics and development of resistance to commonly prescribed antibiotics, hence, antimicrobial susceptibility testing is imperative in selecting therapeutic options.

Keywords: Resistance; Bactericidal; Antibiotics; Susceptibility; Sensitivity; Multi-Drug; Inhibitory

1. Introduction

Antibacterial (Antibiotics) are either bactericidal or bacteriostatic (cytotoxic or cytostatic) to the bacteria, allowing the body's natural defenses, such as the immune system, to eliminate them. They often act by inhibiting the synthesis of a bacteria cell, synthesis of proteins, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), by a membrane disorganizing agent, or other specific Actions [1].

Antibiotics were considered a magic bullet that selectively target bacteria that were responsible for disease causation, but at the same time would not affect the host [2]. The development of Multidrug Resistance (MDR) is a complicated issue which has become an international dreadful concern. To decrease the rise and spread of MDR, cooperative efforts are requisite because diseases which were curable earlier are becoming major causes of deaths in this era [3].

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Antibacterial resistance is the ability of bacteria to resist the effects of medication that once could successfully treat the bacteria [4]. Resistance is the term referred to as the insensitivity of bacteria to antibacterial drug when compared with other isolates of the same species [5]. Multidrug resistance (MDR) is defined as insensitivity or resistance of a microorganism (bacteria) to the administered antimicrobial medicines (which are structurally unrelated and have different molecular targets) despite earlier sensitivity to it [3].

The Multidrug resistances among members of gram negative bacilli (*E.coli, Salmonella typhi and Shigella species*) at a terrifying rate to different antibacterial drugs have become a public health threat all over the world [6].

Antibacterial drugs generally act on the bacteria either by inhibiting a metabolic pathway like nucleotide synthesis which in turn leads to the inhibition of DNA/RNA synthesis and further protein synthesis and disruption of the cell membrane or by competing with the substrate of any enzyme involved in cell wall synthesis [7]. Bacteria have evolved a multitude of mechanisms to overcome the effectiveness of drugs, thereby surviving exposure to the drugs [7].

Due to the pacing advent of new resistance mechanisms and decrease inefficiency of treating common infectious diseases, it results in failure of bacteria response to standard treatment, leading to prolonged illness, higher expenditures for health care, and an immense risk of death. Among all the capable infecting bacteria, clinical isolates of these bacteria have employed high levels of multidrug resistance (MDR) with enhanced morbidity and mortality; thus, they are referred to as “superbugs” [6].

Although the development of multidrug resistance (MDR) is a natural phenomenon, extensive rise in the number of immunocompromised conditions, the inappropriate use of antibacterial drugs, inadequate sanitary conditions, inappropriate food handling, and poor prevention and control practices contribute to emergence and encourage the further spread of MDR [8].

Resistant genes in most bacteria are frequently found in extra chromosomal elements known as R. Plasmid. Clinical isolates of enteric bacteria (*Escherichia coli*, *Salmonella typhi* and *Shigella* species) are naturally resistant to many widely used antibiotics, making chemotherapy difficult to achieve [9]. Multiple antibiotic resistances (MAR) indexing has been shown to be a cost effective and valid method of bacteria source tracking.

Multiple antibiotic resistance indexes is calculated as the ratio of number of resistant antibiotics to which organism is resistant to total number of antibiotics to which organism is exposed [10]. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used. MAR index of 0.4 or higher is associated with human fecal source of contamination [11]. MAR index values > 0.2 indicate existence of isolate from high – risk contaminated source with frequency use of antibiotics while values ≤ 0.2 show bacteria from source with less antibiotics usage High MAR indices mandate vigilant surveillance and remedial measures [10].

At present, the challenge of multidrug resistance in *Escherichia coli, Salmonella typhi* and *Shigella* species from clinics is at alarming rate owing to the indiscriminate use of antibiotics, poor sanitation, prolong hospital stay which may result to nosocomial infection and previous treatment with broad spectrum antibiotics, case of immunocompromised patients and biochemical mechanisms operating within the bacteria. The epidemiology of multidrug resistance of these isolates prompt the needs to unravel the threat posed on human population for empiric management.

The emergence of multidrug-resistant (MDR) bacteria is one of the stagiest clinical and biological phenomena identified over the past century [4]. Since the discovery of antibiotics in the 1920s, each introduction of a new antibiotic class has been followed by the advent of resistance, in one way the number of identified resistance mechanisms, and their abundance, has increased at an alarming rate [12]. Molecules with the ability to kill or inhibit bacteria have likely been around since the first single-cell entities emerged, but the recent pace of evolution is still remarkable, indicating the key role for selective pressure in this process [13].

There have been arguments about where this pressure is most intense, in the clinic, in agriculture, or in the environment but it is likely that within each of these domains, pressure is being exerted that is driving resistance [14]. Particular properties of the genes and mutations involved in resistance explain some aspects of their ability to evolve rapidly. Resistance determinants are largely components of the bacteria “accessory” genome where genetic flexibility is more readily tolerated and such determinants are often “mobile,” enhancing their ability to spread through infectious mechanisms [14].

Hence, within species and between species evolution is exerting an influence. A key feature of the antibiotic resistance era has been the recent emergence of highly successful strains or clones within bacteria species that have the ability to be resistant without any obvious impact on fitness [15].

Indeed, there is evidence that such strains can be more aggressive in terms of their ability to transmit and cause clinical disease. Resistant strains have been identified in many of the common bacteria pathogens, such as *Escherichia coli* and *Salmonella enterica* [16].

Other groups have driven the emergence of newly recognized pathogens, including *Shigella* specie sand *Salmonella typhi* [17]. Some of these strains appear to have adapted to exploit modern human-created niches, such as the healthcare system or food chain. Some have the potential for global spread. Here, with a focus on the enteric bacteria, this study explores some of the factors driving the successful emergence of multidrug resistance and the associated successful resistant strains [17]

Primary Resistance occurs when the organism has never encountered the drug of interest in a particular host. Secondary Resistance, also known as "acquired resistance," this term is used to describe the resistance that only arises in an organism after an exposure to the drug [18]. It is further be classified as follows. Intrinsic resistance which refers to the insensitivity of all bacteria of a single species to certain common first-line drugs, which are used to treat diseases based on the clinical evidence of the patient. It is also known as multidrug resistance (MDR) [15].

Extensive resistance defines the ability of organisms to withstand the inhibitory effects of at least one or two most effective antibacterial drugs. Also termed as XDR, this seemed to arise in patients after they have undergone a treatment with first line drugs, for example, XDR-TB resistance against fluoroquinolone [19].

Clinical resistance is defined by the situation in which the infecting organism is inhibited by a concentration of an antibacterial agent that is associated with a high likelihood of therapeutic failure or reappearance of infections within an organism due to impaired host immune function. In other words, the pathogen is inhibited by an antimicrobial concentration that is higher than could be safely achieved with normal [15].

Bacteria can also become resistant through mutations that make the target protein less susceptible to the agent. Some resistance is mainly (but not exclusively) due to mutations in the target enzymes, DNA topoisomerases. Whether resistance of this type is easily transferred to other cells on plasmids depends on the mode of the drug's action [20].

Cell wall in bacteria plays a crucial role in their survival; drugs inhibit the cell wall synthesis by binding with the peptidoglycan layer in bacteria, thus, blocking the cell growth and division [21]. These microbes undergo certain chromosomal mutations or exchange of extra chromosomal DNA elements through conjugation or transformation (horizontal gene transfer) such as in *Escherichia coli* which can cause alteration in the cell membrane composition resulting in decreased permeability and uptake of drugs into the cell [3]. Altered membrane composition also leads to lack of active target sites for the drugs to bind. Mutations in the genes encoding for the target cause modifications at the molecular level and retain cellular function by reducing susceptibility to inhibition [3].

Another mechanism of MDR was found to be an over expression of drug target enzymes leading to target bypass due to modification in certain metabolic pathways which causes production of alternate target molecules and interference in some protein synthesis. This can influence the access of drugs to the target sites [22].

A common resistance mechanism for antibiotics of natural origin, such as Aminoglycosides usually in the periplasm, Genes coding for these inactivating enzymes (enzymatic inactivation of drugs) can easily produce resistance as additional genetic components on plasmids [23]. Inactivation or enzymatic degradation of antibacterial by hydrolysis of ester or amide bonds (such as resistance to β-lactams due to β-lactamases, etc.) and chemical alterations of these compounds by acetylation, phosphorylation, adenylation, glycosylation, and hydroxylation have also become increasingly perceived as causes of MDR. The resistant strains of these clinical isolates have developed the ability to oxidize or reduce the antibacterial compounds to prevent their interaction with the respective targets [24].

Multidrug resistance (MDR) mediated by drug efflux pumps remains the predominant mechanism of multidrug resistance. the over expression of genes encoding for ATP-binding cassette (ABC) transporter membrane proteins (e.g., P-glycoprotein (Pgp)), also known as the multidrug efflux pumps which are responsible for the export or expulsion of drugs out of the cell, usually generates MDR and continues cellular functions without any interference [25]. Over

expression of P-glycoprotein, membrane or multidrug resistant proteins (MRP), affects the fluidity and permeability, leading to an ATP-dependent efflux of the antibacterial and decreasing their intracellular concentration [26].

Multidrug resistance (MDR) is also employed by cancer cells, which limits the long-term use of chemotherapy. An insight into the mechanisms involved in the chemo resistance, which can occur either at the beginning of the therapy (innate) or during the course of treatment, reveals that the cancer cells exhibit over expression of certain multidrug resistance proteins (e.g., MRP and Pgp) which induce DNA repair mechanism, inhibit apoptosis, alter drug targets, and modify cell membrane composition as well as promoting an increased efflux of drugs preventing proper diffusion into the cells [27].

The antibiotic susceptibility of bacteria cells is affected by their physiological states. One important consequence of this phenomenon is the occurrence of "persister" cells. Thus, it was discovered early that even high concentrations of antibiotics do not kill all of the bacteria population, leaving behind a persister population that is genetically identical with the susceptible cells [28]. When biofilms were found more resistant to most antibiotics, there were initial attempts to interpret this finding on the basis of a more limited diffusion of drugs through the biofilm structure. However, this mechanism cannot produce large increases in resistance [28].

Although there are interesting data that link drug resistance in some bacteria biofilms to the production of periplasmic β-(1–3)-glucans and an efflux system, It is difficult, at least at present, to explain the extensive antibiotic resistance of biofilms on the basis of alteration of all cells in the population [28].

It is therefore attractive to explain such resistance as the result of the presence of a large number of per sister cells in the biofilm population [28]. The presence of per sisters is now thought to be an example of the strategy whereby bacteria naturally generate mixtures of phenotypically different populations, so that one of them can be advantageous to a changing environmental demand [29]. Per sisters limit the efficacy of antibiotic therapy, and we note that a recent single-cell study has identified an antibiotic-susceptible phase in the life cycle of typical persister cells [30].

Antibacterial resistant may be acquired by emergence of resistance in endogenous flora, or by acquisition from other patients and the environment. Emergence from endogenous flora has been frequently reported among gram-negative organisms with inducible beta-lactamases and in Pseudomonas aeruginosa [31]. A recent important example is the emergence of glycopeptides intermediate MRSA (GISA). These strains have usually developed in patients with persistent or relapsing MRSA infection associated with foreign bodies such as hemodialysis catheters which have not been removed, and after prolonged vancomycin therapy [31].

An organism may be transmitted from another patient in an outbreak setting where an increased number of cases is observed, or as endemic transmission where a continuing, stable, number of cases occur. A patient may also acquire a resistant organism, such as an aminoglycoside resistant *S. marcescens*, and the genetic resistance elements may be transferred to other colonizing flora resulting in multiple resistances in other species such as *K. pneumoniae* or *E. coli* [32].There is overlap between these two types of acquisition. Once a patient develops endogenous resistance, the strain may be transmissible to other patients, leading to an outbreak or endemic colonization or infection with the resistant organism. Bacteria which may initially cause outbreaks, such as MRSA and VRE, may subsequently become endemic in the facility [31].

Some of the types of antibiotic misuse in clinical practice include unjustified prescription (in such conditions as diarrhoea and the common cold), under-prescription (for example, in urinary tract infections and sexually transmitted diseases), under dosing, and short duration.

Antibacterial prescription in many developing countries is almost entirely empirical and based on surveillance data obtained from locations or at a time that it is unlikely to be relevant to the ensuing situation [33]. Broad-spectrum agents are frequently employed because susceptibility data is unavailable. In addition, the rising number of HIV-positive people increases selective pressure for resistant organisms by increasing the need for prophylactic and curative antimicrobial use [34].

In a study designed to determine the health-seeking practices of educated Nigerians, a cohort of university undergraduates was followed for one year. The students were all entitled to free medical care provided by physicians at the University Health Center and drug supply from an attached pharmacy. Within the year, of 43 students who had diarrhea, only 16 percent sought treatment at the health center [35].

Twelve percent did not seek treatment at all but 72 percent resorted to self-medication, at their own expense, even though they could obtain medical care at no cost. Furthermore, 80 percent of the self-medicators took at least one antibacterial medicine and 45 percent took more than one antibacterial. Clearly, factors other than cost and health care availability influence antibiotic misuse [36].

Even when antibiotic prescription and use is optimal, sub-therapeutic dosing and consequent resistant selection can arise from poor quality antibiotic preparations. Multiple reports have described the dispensing of medicines in Nigeria that contain as little as half of their label content. Some of the medicines are counterfeit—they were intentionally formulated with less than the stated content but significant proportions contain degraded drugs [37].

Degradation of heat- and moisture labile antibiotics occurs very readily in tropical developing countries where ambient temperatures may approach 40°C and humidity is high enough to distort capsule shells and soften tablets. The packaging of many pharmaceuticals may be insufficient to protect them in tropical developing countries, and in some cases it may be desirable to reformulate them for this environment or modify shelf life recommendations [38].

One rationale for employing uniform shelf lives in temperate and tropical countries is that labels indicate that medicines are to be stored under controlled conditions. Indeed, local regulations usually specify that pharmacy premises in the tropics must be air-conditioned. In reality, frequent power cuts may mean that the air is only intermittently conditioned, warehouses and unapproved premises are almost never air-conditioned, and imported drugs may depreciate for many months at ports [39].

2. Materials and methods

The following materials were used in the experiment, Sterile bijou bottles, flat bottom flask, Graduated measuring cylinder, Conical flask, Beaker, Test tubes, Glass slides, Digital weigh balance, Standard wire loop, Bunsen burner, Glass slides, Light microscope, Maker, Incubator, Refrigerator, Distilled water, Aluminum foil paper, Sterile cotton wool, Autoclave, Masking tape, Hand gloves, Alcohol, Detergent, Test tube rack. Media used are Nutrient Agar (NA), Salmonella-Shlgella agar (S-S Agar), Eosin Methylene Blue Agar (EMB), Muller Hilton Agar and sensitivity disc. All the media were prepared as recommended by the manufacturer.

A total of 32 pre-identified clinical isolates of gram negative bacilli (*Salmonella typhi*, *Shigella* species and *Escherichia coli*) were obtained from Institute of Medical and veterinary Laboratory Science Vom during March, 2019 to May, 2019 and preserved on nutrient agar for further identification. Isolates sub-cultured from nutrient agar slants onto Eosin methylene blue (EMB) and *Shigella* Salmonella agar (SS agar) medium were identified by a combination of colonial morphological characteristics, gram stain, and biochemical characteristics. Antibiotic susceptibility pattern of the isolates was done after they were re-identified. The E. coli produces distinctive metallic green sheen on EMB while Salmonella appears pale with black centers and *Shigella* appears pale on SS-Agar

Biochemical test was carried out to identify the bacteria. The types of biochemical reactions each organism undergoes act as a "thumbprint" for its identification. The group of test used mainly to identify the members of enterobacteriaceae (*Shigella* species, *Salmonella typhi* and *Escherichia coli*) includes Indole test, Methyl red test, Voges-proskauer test, and Citrate test commonly refers to as IMViC test.

Indole is a component of the amino acid tryptophan. Some bacteria have the ability to break down tryptophan for nutritional needs using the enzyme tryptophanase. When tryptophan is broken down, the presence of indole can be detected through the use of kovac's reagent. Kovac's reagent, which is yellow, reacts with indole and produces a red color on the surface of the test tube. Indole-Positive reaction will give red color indicating E. coli.

Principally to test the ability of the organism to produce acid such (lactic, acetic, succinate and formic acid)as end product from glucose fermentation, it is a qualitative test for acid production. Positive test will give bright red color.

To determine the ability of the isolates to produce neutral end product acetoin from glucose fermentation, a positive reaction gave pink or red.

Simmons Citrate agar was carried out to study the ability of an organism to utilize citrate presence in simon's medium as a sole source of carbon. If the citrate is utilized, the resulting growth will produce alkaline products changing the colour of the medium from green to blue. Green color will indicate negative reaction for *E.coli*.

Grease or oil free slides are essential for the preparation of these smears. Grease or oil from the fingers on the slides is removed by washing the slides with soap and water. The slides were wiped with alcohol. After cleaning, allowed to dry and were place on laboratory towels until ready for use. Drawing a circle on the underside of the slide using a glassware-marking pen may be helpful to clearly designate the area in which the smear will be prepared. The slides were labeled with the initials letter of the name of the isolates on the edge of the slide.

Isolate suspensions in broth: With a sterile cooled loop a loopful of the broth culture was placed on the slide. Spread by means of circular motion of the inoculating loop to about one centimeter in diameter. The spreading was done evenly to avoid excessive spreading which may result in disruption of cellular arrangement. A satisfactory smear was made to allow examination of the typical cellular arrangement and isolated cells.

With a sterile cooled loop, a drop of sterile water was placed on the slide, Sterilized and allowed to cool again. The loop was used to pick up a very small sample of the isolate each colony and gently stirred into the drop of water on the slide to create an emulsion. The smear was firmly adhered to the slide, and allows the sample to more readily take up stains. The smear was allowed to air dry. After the smear has air dried, the entire slide was passed through the flame of a Bunsen burner two to three times with the smear-side up.

The heat fixed smear on staining tray was gently flooded with crystal violet and allowed to stand for 1 minute, Tilt the slide slightly and gently rinse with distilled water using a wash bottle. The smear was gently flooded with Gram's iodine and allowed to stand for 1 minute, tilt the slide slightly and gently rinse with distilled water using a wash bottle. The smear appeared as a purple circle on the slide. It was decolorized using 95% ethyl alcohol. Tilt slightly and alcohol was applied drop by drop for 5 to 10 seconds until the alcohol runs almost and was immediately rinsed with water. The slide was gently flooded with safranin to counter-stain and allowed to stand for 45 seconds, Tilt slightly and gently rinsed with distilled water using a wash bottle, the slide was allowed to air dry and viewed using a light-microscope under oil-immersion.

Antibiotic susceptibility test was determined by the disc agar diffusion method according to Clinical and Laboratory Standards Institute [40]. Agar diffusion assays was performed on Muller Hilton media. The following antibiotics: Amplicin (PN: 30 mcg), Gentamicin (GEN: 10 mcg), Streptomycin (STR: 30 mcg), Septrin (SXT: 30 mcg), Nalidixic acid (NAL: 30 mcg), Ciprofloxacin (CIP: 10 mcg), Augmentin (AU: 30 mcg), Reflacin (PEF: 10 mcg), Tarivid (OFX: 10mcg), and Ceporexin (CEP: 10 mcg) were used for antibiotic susceptibility test. After 24 hours of incubation at 370C, zone of inhibition was measured and record base on standard zone of inhibition interpretation chart by World Health Organization [41].

3. Result

A total of 32 pre-identified clinical isolates was obtained from Institute of Medical and veterinary Laboratory Science Vom. Morphological and biochemical identification revealed (34.38%) *E.coli*, (31.25%) *Salmonella typhi*, (31.25%) *Shigella* species while (3.13%) were *Klebsiella*.

Table 1 Cultural, Morphological and Biochemical characteristics of the Isolates

S/No	Isolates	Shape	Colour	Morphology	Gram reaction	Indole test	Citrate test	Methyl red	Voges proskauer
1.	*Salmonella typhi*	Circular	Pale	Rod	-	-	+	+	-
2.	*Shigella species*	Circular	Pale	Rod	-	+	-	+	-
3.	*E.coli*	Irregular	Green Metallic Sheen	Rod	-	+	-	+	-

Key: - negative, +positive

Table 1 shows the cultural characteristics of the isolate as confirmed using conventional standard biochemical test and gram staining reaction. *Salmonella typhi* and *Shigella* speciesi appear pale on S-S agar, both are methyl red positive,

voges-proskauer negative. *Shigella* is indole positive and citrate negative. *E.coli* appeared irregular with green metallic sheen on EMB agar; it is indole positive, methyl red positive and negative for others.

Table 2 show the susceptibility pattern of *Salmonella typhi* to each antibiotic used. Amplicin 9(90%) demonstrated the highest levels of resistance. Isolates were most sensitive to Tarivid 9(90%). Nearly all the isolates had Multiple Antibiotic Resistance (MAR) index greater than 0.2.

Table 2 Antibiotic Susceptibility Pattern of *Salmonella typhi*

Antibiotics (mcg)		Sensitivity No. (%)	Resistance No.(%)	MARI-index
OFX	10	9(90)	1(10)	0.1
PEF	10	7(70)	3(30)	0.3
CPX	10	2(20)	8(80)	0.8
AU	30	7(70)	3(30)	0.3
GEN	10	8(80)	2(20)	0.2
S	30	6(60)	4(40)	0.4
CEP	10	3(30)	7(70)	0.7
NAL	30	1(10)	9(90)	0.9
SXT	30	3(30)	5(50)	0.5
PN	30	0	9(90)	0.9

KEY: OFX-Tarivid, PEF-Reflaxin, CPX-Ciprofloxacin, AU-Augmentin, S-Streptomycin, CEP-Ceporexin, NAL-Nalidixic acid, SXT-Septrin, GEN-Gentamycin, PN-Amplicin, mcg-Microgram, MARI-multi antibiotic resistance index, %-Percentage

Table 3 showed the susceptibility pattern of *Shigella* species. Nalidixic acid 9(90%) demonstrated the highest levels of resistance followed by septin 7(70%) with streptomycin 6(60%). Isolates were most sensitive to Tarivid 8(80%), Ciprofloxacine 9(90%), Augmentin 8(80%) and Reflacin 9(90%). Nearly all isolates 6 (60%) had Multiple Antibiotic Resistance (MAR) index greater than 0.2 and was resistance to two antibiotic tested.

Table 3 Antibiotic Susceptibility Pattern of *Shigella* Species

Antibiotics (mcg)		Sensitivity		Resistance		MARI-index
		NO	%	NO	%	
OFX	10	8	80	2	20	0.2
PEF	10	9	90	1	10	0.1
CPX	10	9	90	1	10	0.1
AU	30	8	80	2	20	0.2
GEN	10	6	60	4	40	0.4
S	30	4	40	6	60	0.6
CEP	10	5	50	5	50	0.5
NAL	30	1	10	9	90	0.9
SXT	30	3	30	7	70	0.7
PN	30	1	10	9	90	0.9

KEY: OFX-Tarivid, PEF-Reflaxin, CPX-Ciprofloxacin, AU-Augmentin, S-Streptomycin, CEP Ceporexin, NAL-Nalidixic acid, SXT-Septrin, GEN-Gentamycin, PN-Amplicin, mcg- Microgram, MARI- multi antibiotic resistance index %- Percentage.

Table 4 demonstrates the susceptibility pattern of *E.coli*. High resistance rate to Ceporexin 8(73%), Septrin 6(55%), gentamycine 9(82%) Nalidixic acid 7(64%) and Amplicin 9(82%). Also showed high susceptibility to Tarivid 7(64%), Ciprofloxacin 9(82%), Sreptomycin, 6(55%), Augmentin 8(73%) and Reflacin 7 (64%). Nearly all isolates (82%) had Multiple Antibiotic Resistance (MAR) index greater than 0.2 and was resistance to four antibiotics tested.

Table 4 Antibiotic Susceptibility Pattern of *E. coli*

Antibiotics	**(mcg)**	**Sensitivity**		**Resistance**		**MARI-index**
		NO	**%**	**NO**	**%**	
OFX	10	7	64	4	36	0.4
PEF	10	7	64	4	36	0.4
CPX	10	9	82	2	18	0.2
AU	30	8	73	3	27	0.3
GEN	10	2	18	9	82	0.8
S	30	6	55	5	45	0.5
CEP	10	3	27	8	73	0.7
NAL	30	4	36	7	64	0.6
SXT	30	5	45	6	55	0.5
PN	30	2	18	9	82	0.8

KEY: OFX-Tarivid, PEF-Reflaxin, CPX-Ciprofloxacin, AU-Augmentin, S-Streptomycin, CEP-Ceporexin, NAL-Nalidixic acid, SXT-Septrin, GEN-Gentamycin, PN-Amplicin, mcg-Microgram, MARI-multi antibiotic resistance index, %-Percentage

4. Discussion

Multi antibiotic resistance index is helpful in analyzing health risk as well as to check the extent of antibiotic resistance. MAR index analysis in this study is used to differentiate isolates from different sources using antibiotics that are commonly used in treatment of infectious cases, compared to other methods of bacteria source tracking, it is cost effective, rapid and easy to perform. A MAR index greater than 0.2 and above indicate high risk source of contamination where antibiotics are often used.

This study has revealed interesting findings concerning multidrug resistance profiles among enteric bacteria pathogens (*Salmonella typhi, Shigella* species and *E.coli*) isolated from clinical samples. Although bacteria resistance results primarily as a consequence of selection pressure placed on susceptible bacteria by the use of therapeutic agents, a variety of socio-medical factors such as multiplicity of sources, substandard medicine, antibiotic prescription by health professionals and dissemination also contribute to the emergence and spread of multidrug resistance.

In this study, a significant number of *E.coli* studied had multi antibiotic resistance index (MAR-index) greater than 0.2 and above with multidrug resistance to Septrin, Nalidixic acid, Amplicin, Ceporexin and Gentamycin indicating their source to be from where antibiotics are commonly used. This finding agree with the work of [42]. which half 0f the isolates tested in their study exhibit multidrug resistance character suggesting the existence of greater frequency of MDR in the study area.

Following the analysis of the trend in susceptibility pattern of *Shigella* species, multi antibiotic resistance index was also greater than 0.2 and above with low susceptibility rate of the antibiotics tested. High rate of resistance to more than two antibiotic tested was also noted. This is in accordance with the study carried by [9] which reported that clinical isolates of enteric bacteria (*E.coli, Salmonella typhi and Shigella species*) are naturally resistant to many widely used antibiotics, so chemotherapy is often difficult. The result of this finding revealed that *Salmonella typhi* was susceptible to nearly all the antibiotics and was resistance to only three antibiotics tested. However, this is greater compared to the findings of [43] in which only 50% was recorded in their study of clinical management of typhoid suggesting the lower cases of multidrug resistance in the study area.

5. Conclusion

Rapid increase of severe systemic infections and the spread of resistant bacteria are indisputable facts. Inadequacy of available antibacterial compels continuous development of newer drugs. Moreover, various awareness programs which should facilitate their appropriate use to reestablish dominance over diseases must be implemented. MDR is an unavoidable natural phenomenon, posing a serious worldwide menace to public health. A cooperative action at global level is a must to combat MDR. Pathogens tend to adopt various resistance mechanisms to survive the unfavorable conditions. Improved knowledge of molecular mechanisms controlling MDR should facilitate the development of novel therapies to combat these intransigent infections and will help cultivate a deeper understanding of the of bacteria resistance.

Recommendation

The development of MDR is a complicated issue which has become an international dreadful concern. To decrease the rise and spread of MDR, cooperative efforts are requisite because diseases which were curable earlier are becoming major causes of deaths in this era. Moreover, focusing on areas which are susceptible to inappropriate use of antibacterial by implementation of antibiotic stewardship (defined as coordinated interventions designed to improve and measure the appropriate use of antibacterial) is the need of the hour. Priority should be given to education, directed at distributors and consumers as well as prescribers of antibiotics, infection control to prevent the dissemination of resistant strains, quality assurance of antibiotics and other medicines, and the institution of functional and sustainable laboratories for antibiotic resistance surveillance.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical Approval

Ethical approval was obtained from the Benue State Health Management Board with an Issuance of an ethical clearance certificate from the ethics committee. All participants were informed of the details of the study before samples were collected.

Statement of Informed Consent

Informed consent was obtained from all individuals who participated in this study.

References

[1] Levy, S. B. and Marshall, B., (2004). Antibacterial resistance worldwide, Causes, challenges and responses10:122–129

[2] Aminov RI. (2010).Origins and evolution of antibiotic resistance 1:134.

[3] Singh, V. (2013). Antimicrobial resistance in Microbial Pathogens and Strategies for Combating them vol. 1:291–296

[4] World Health Organization, (2018). Antimicrobial Resistance, Global Report on Surveillance, Geneva, Switzerland.

[5] Loeffler, J. and Stevens, D. A., (2009). Antifungal drug resistance vol. 36:31–41

[6] Nikaido E and Zli H. (2009). Efflux-mediated drug resistance in bacteria vol. 69:1555–1623

[7] Friedman, N. D., Temkin, E. and Carmeli, Y., (2016). The negative impact of antibiotic resistance. Clinical microbilogical infectious diseases. 22(5): 416-422

[8] Nikaido, H., (2009) Multidrug resistance in bacteria vol. 78:119–146

[9] Dubois, V. Arpin, C. Melon, M. Melon, B. Andre, C. Frigo, C. and Quentin, C. (2001). Nosocomial outbreak due to a multiresistant strain of Pseudomonas aeruginosa P12: Efficacy of Cafepime-Amikacin therapy and analysis of Beta- Lactam resistance. Journal of Clinical Microbiology 39(6): 2072-2078

[10] Joseph, A. A., Micheal, S. O., Olokoba, L. B. and Olokoba, A. B., (2017). Multiple antibiotic resistance index of Escherichia coli isolates in a tertiary Hospital in South-West Nigeria

[11] Mishra, M., Patel, A. K., and Behera, N., (2013). Prevalence of multidrug resistance E.coli in the river Mahanadi of sambalpur vol1 (5):239-244.

[12] Dzidic, S., Suskovic, J. and B. Kos, (2008). Antibiotic resistance mechanisms in bacteria: biochemical and genetic aspects vol. 46, no. 1, pp. 11–21.

[13] Munita, J. M., and Arias, C. A., (2016). Mechanisms of antibiotic resistance. Microbiol. Spectr; 4, VMBF-0016-2015

[14] MacLean, R. C., and SanMillan, A., (2015). Microbial evolution: Towards resolving the plasmid paradox 25:764–767.

[15] Harris, S. R., (2010). Evolution of MRSA during hospital transmission and intercontinental spread 327:469–474.

[16] Kingsley, R. A., (2016). Epidemic multiple drug resistant Salmonella typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype 19:2279–2287.

[17] Elliott B, Androga GO, Knight DR, Riley TV (2017) Clostridium difficile infection: Evolution, phylogeny and molecular epidemiology. 49:1–11.

[18] Freeman, J., Vernon, J., Pilling, S., Morris, K., Nicolson, S. Shearman, S., Clark,E., Palarios-Fabrega, J. A. and Walcox , M., (2020). Pan-European longitudinal surveillance of antibiotic resistance among prevalent Clostridium difficile Ribotypes' study group. Five year Pan-European longitudinal surveillance Clostridium difficile Ribotype prevalence and antimicrobial resistance: the extended closer study. European Journal of Clinical Microbiology of Infectious Diseases; 39(1)169-177

[19] Pitout, J. D. and DeVinney, R., (2017). Escherichia coli ST131: A multidrug-resistant clone primed for global domination 6:195.

[20] Hooper, C. D. and Jacoby, A. G., (2016). Mechanism of drug resistance: quinolone resistance, National Library of Medicine: 1354 (1): 12-31

[21] Alekshun, M. N. and Levy, S. B., (2007). Molecular mechanisms of antibacterial multidrug resistance vol. 128:1037–1050,

[22] Kaminski, S. G., Li, S. and He, X., (2013). Using Aspergillus nidulans to identify antifungal drug resistance mutations vol. 13, 288–294,

[23] Davies, J., and Davies, D., (2010). Origin and evolution of antibiotic resistance Microbiol. Mol. Biol. Rev.74(3):417-433.

[24] Chethana, G.S., K. R. Hari venkatesh, F. Mirzaei, and S. M. Gopinath (2013) Review on multidrug resistant bacteria and its implication in medical sciences Journal of Biological Scientific Opinion, vol. 1:32–37

[25] Levy, S. B., Chadwick, D. J. and Goode, J., (2007). Antibiotic Resistance: An Ecological Imbalance, in Ciba Foundation Symposium Vol 1:20

[26] Peleg, A. Y., Seifert, H. and Paterson, D. L., (2008). Acinetobacter baumannii: Emergence of a successful pathogen. 21:538–582.

[27] Kunjachan, S. Rychlik, B., Storm, G., Klessling, F. and Lammers, T., (2014). Multidrug Resistance; Physiological principles and Nanomedical solutions 65(0): 1852-1865.

[28] Lewis, K., (2005). Persister cells and the riddle of biofilm survival. Biochemistry (Moscow) 70:267- 274.

[29] Udo, E.E.,Al-Sweih, N. Dhar, R. Dimitrov, T.S. Mokkadas, E,M, Johny, M.Al-Obaid, I.A. Gomaa, H.H. Mobasher, L.A. Rotimi, V.O. and Al-Asar A. (2007) Survellance of antibacterial resistance in Staphylococcus aureus isolated in Kuwaiti Hospitals. Medical Principles and Practice 17 (1): 71-75

[30] Gefen, O., Gbay, C., Muncuoglu, M., Engel, G. and Balaban, N. Q., (2008). Single-cell protein induction dynamics reveals a period of vulnerability to antibiotics in persister bacteria; Proc Natt Acad Sci USA. 105: 6145-6149.

[31] McGowan, J. E. JR., (2001). Cost and benefit in perioperative antimicrobial prophylaxis methods for economic analysis. Rev. Infect Dis; 13(10) 5879-5889.

[32] Raksha, Y., Srinivasa, H., and Baijayani, M., (2009). Magnitude of drug resistance in shigellosis 27:358-360.

[33] Paredes, P. de la Pena, M., Flores-Guerra, E, Diaz, J.and Trostle J. (1996). Factors Influencing physicians' prescribing behaviour in the treatment of childhood diarrhoeal: knowledge may not be the clue. Social Sciences and Medicine. 42: 1141-1153

[34] Gordon, M. A., (2002). Non-typhoidal Salmonella bacteraemia among HIV infected Malawian adults: High mortality and frequent recrudescence. 16:1633–1641.

[35] O'Connor, S. Rifkin, D., Yang, Y.H. Wang, J.F., Levine O.S and Dowell, S.F. (2021). Physician control of pediatric antimicrobial use in Beinjing, China and it's rural environs. Pediatric infectious disease Journal , 20:679-684

[36] Okeke, I. N., Lamikanra, A. and Edelman, R., (1999). Socioeconomic and behavioural factors leading to acquired bacterial resistance to antibiotics in developing countries. Emerging Infectious Diseases (5):18-27.

[37] Okeke, I.N., Fayinka S.T., Lamikanra A. (2000). Antibiotic resistant trends in Escherichia coli from apparently healthy Nigeria students (1986-1998). Emerging infectious diseases. 6: 393-396

[38] Aboderin, O.A. Abdu, A. Odetoyin, B.W. Lamikanra, A. (2009). Antimicrobial resistance in Escherichia coli strains from Urinary tract infections. Vol. 101 (12): 1268-1273

[39] Mthembu, P. T., Zishiri, T. O., and El-Zowalaly, E. M., (2019). Molecular detection of multidrug – resistant Salmonella isolated from livestock production system in South – Africa. Infectious and Drug Resistance12 pp3537- 3548.

[40] Clinical and Laboratory Standards Institute (CLSI) (2017)Performance Standards for Antimicrobial Disk Susceptibility Tests, 12th Edition, Clinical and Laboratory Standards Institute, Wayne PA.

[41] World Health Organization, Geneva, Switzerland, (2014). Antimicrobial Resistance Global Report on Surveillance,

[42] Subramani S, Vignesh S (2017).MAR index study and MDR character analysis of a few golden staph isolates, Asian journal of pharmaceutical life science 2(2) 151-153.

[43] Shetty, A.K., Shetty, I. N., Furtado, Z.V., and Antony, B., Boloor, R., (2012). Antibiogram of Salmonella isolates from blood sample with emphasis on nalidixic acid and chloramphenicol susceptibility in tertiary care hospital in coastal Karnataka 4(2) 7-17.