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Survey of Multidrug Resistant *Salmonella enterica serovar* Typhi from Patients with Pelvic Inflammatory Disease attending some hospitals in Niger State, Nigeria

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Abstract
Drug resistance, especially multidrug resistance by microorganisms, particularly bacteria is on the increase and has been considered as a major health challenge worldwide. This study was conducted to isolate multidrug resistant (MDR) Salmonella typhi associated with patients with pelvic inflammatory disease (PID) attending three General Hospitals in Niger State. A total of 390 samples of endocervical swabs (ECS) and urine samples were collected using sterile swab sticks and sample containers from patients atending General hospital Bida, Suleja and Kontagora. Screening for the presence of Salmonella typhi was done using streak method. Isolates of Salmonella typhi were identified through Gram staining and other biochemical tests. The antibiotic susceptibility profile of the isolates to ten (10) commonly prescribed antibiotics was determined using Kirby-Bauer disc diffusion method on Mueller-Hinton agar. The result showed 240 (62%) of the 390 samples were positive for bacterial infections. Specifically, 50 (20.8%) of the 240 bacterial positive samples from both ECS and urine were positive for S. typhi .The antibiogram showed that 18 (36.0%) S. typhi isolates out of the 50 S. typhi isolates, expressed multidrug resistant characteristics, and were resistant to more than three (3) classes of antibiotics. The multidrug resistant S. typhi exhibited resistance to: Ofloxacin, Nalixidic acid, Augmentin, Cephalexin, Perfloxacin and Streptomycin. The results of this study confirmed the presence of multidrug resistant S. typhi in Niger State, hence there is a need for public health workers, to create awareness on the misuse of antibiotics, to prevent and curtail treatment failure due to antibiotic resistance.
Keywords: Pelvic inflammatory disease, Urogenital samples, Urogenital pathogens, Multidrug resistant Salmonella typhi

INTRODUCTION

Drug resistance is the ability of an organism to withstand the effect of a drug (particularly an antibiotic or a group of antibiotics). Emergence of drug resistance among the most important bacterial pathogens is recognized as a major public health threat affecting humans globally (Munita and Arias, 2016). Multidrug resistant organisms have emerged not only in the hospital environment but are now often identified in the community settings, suggesting that reservoirs of antibiotic resistant bacteria are present outside the hospital (Munita and Arias, 2016).

However, most of these multidrug resistant bacteria, belonging to various genera such as: *Escherichia, Salmonella, Klebsiella, Pseudomonas, Staphylococcus* and *Proteus* (Meštrović, 2017) are commonly referred to as the urogenital pathogens (Oyedum, 2021). In addition, they are the main causative agents associated with most prevailing bacterial infections, such as pelvic inflammatory diseases (PID) prevalent among women folk in many developed and developing countries of the world are commonly referred to as the (Nikkado *et al.*, 2009; Adekunle, 2012).

Pelvic inflammatory disease (PID) is a disease of various organs (such as the ovaries, fallopian tubes, uterus and endometrium) located in the upper genital tracts of a female (Ahmed 2017). Basically, it is one of the top three prevalent gynaecological problems, associated with female reproductive damages such as; fallopian

tubal blockage, endometriosis and oophoritis, which leads to 10% infertility and 0.5% Occurrence of multi drug resistant urogenital pathogens among females, have led to prolonged hospital stay, treatment failure, morbidity and mortality (Spencer *et al.*, 2014; Centre for Disease Control, 2015). It is therefore imperative to determine the antimicrobial susceptibility of multidrug resistant *Salmonella typhi*, which is one of the predominant urogenital pathogens, associated with most PID in most developing countries. This in turn will enhance the development of new efficient antibiotics and vaccines, in many pharmaceutical industries, to curb the existence and spread of most multi drug resistant bacteria.

mortality among women in the reproductive ages of 25-34 years (Usman, 2016).

MATERIALS and METHODS

Description of the Study Area

The study was conducted in Niger State, Nigeria. Niger State is located in the middle belt zone of the country. It lies between latitude 8˚20′N and 11˚30′N and longitudes 3˚30′E and 7˚20′E. The state has three zones, namely zone A, B and C (Usman, 2016). Zone A is found in the southern part of the state and it comprises Agaie, Bida, Edati, Katcha, Gbako, Lapai, Lavun and Mokwa Local Government Areas; while zone B comprises of Bosso, Chanchaga, Gurara, Kuta, Paikoro Rafi, Shiroro, Suleja and Tafa Local Government Areas and zone C comprises of Agwara, Borgu, Kontagora, Magama, Mariga, Mashegu, Rijau and Wushishi Local Government Areas (as seen in Plate 1). 3 Local Government Areas (comprising of 1 Local Government Area from each zone) were randomly selected for this study.

Plate 1: Map showing the various Local Government Areas in Niger State
Source: Niger State Bureau of Statistics (2010)

Study design

The study was basically cross-sectional. Endocervical swabs and urine samples were collected using stratified sampling method, from every PID patient simultaneously in three (3) government hospitals located in different Local Government Areas, namely; Bida, Suleja and Kontagora. These hospitals in each Local Government Area serve as primary health care centres. This study was conducted from October 2018-October, 2019.

Sample Size

The sample size (n) for each L.G.A was calculated to be 130, using the equation below (Cochran, 1977).

$$n = \frac{\mathbf{T^2 \ x \ P(1-P)}}{\mathbf{m^2}}$$

However, a total of 390 samples were collected from 3 general hospitals located in 3 selected local government areas of Niger state (Bida, Suleja and Kontagora).

Study Population

The study population included two groups namely: Outpatients with any gynaecological problem, who are clinically diagnosed of pelvic inflammatory diseases (but are not yet admitted); and In patients with any gynaecological problem, who are also diagnosed of pelvic inflammatory diseases and admitted (Oseni and Odewale, 2017; Pachori and Kulkarni, 2016).

Inclusion and Exclusion Criteria

Female patients within the age of 15-54years diagnosed of pelvic inflammatory disease (PID) and are attendees of the selected hospitals were recruited for this study. Female patients above 54years and less than 15 years not diagnosed of PID and who are not attendees of the selected hospitals were excluded from this study.

Ethical Clearance

Ethical clearance for this study was sought from the Niger State Hospital Management board, Research and Ethics Committee. The ethical permission reference number for this study is HMD/GHM/136/VOLIII/525

Collection and Transportation of Samples

Sterile flexible swab stick was used for the collection of swabs from the endocervical region of each patient enrolled for the study (Einwalter *et al.*, 2005; Pachori and Kulkarni, 2016; Oseni and Odewale, 2017).

The swabs were removed and submerged into normal saline and were taken to the Microbiology Laboratory of Federal University of Technology, Minna for further analysis (Enwa *et al.*, 2015).

Urine Samples: 5mL of fresh urine was collected from each female patient diagnosed of PID into a universal bottle. The urine samples were transported to the Microbiology Laboratory of Federal University of Technology, Minna under a cold condition of 2°C (Hunter *et al.*, 2013). The urine samples were stored at 4ºC for 24 hours for further analysis (Hunter *et al.*, 2013).

Direct Examination

Saline wet preparation was carried out in order to rule out the presence of *Trichomonas vaginalis* which is characteristically associated with a yellow-green discharge, itching, redness and swelling (Spencer *et al.*, 2014).

Isolation and Identification of Bacteria from the Samples

The endocervical swabs and urine samples were cultured and incubated on Nutrient agar and MacConkey agar at 37ºC for 24h for the isolation of Gram-Negative and Gram-Positive bacteria. Pure culture of each isolate was obtained by continuous sub-culturing using the streak method. The pure isolates were stored on a nutrient agar slant for further identification and characterization (Kolo, 2016).

The isolated bacteria were identified via Gram staining and other conventional biochemical tests such as: Coagulase, Oxidase, Catalase, Citrate, Urease, Indole and Triple sugar test as described by Cheesbrough (2010).

Confirmation of *S. typhi* from the Samples

Confirmation of *S. typhi* was done by culturing and incubating the various samples on *Salmonella- Shigella* medium at 37ºC for 24h. Observation of a black pigmented colony confirmed the presence of *S. typhi*.

Antimicrobial Susceptibility Testing of Isolates

The 0.5McFarland standard was employed in the standardization of the test organisms (Lalitha, 2004). Susceptibility test of the isolates was carried out using Kirby- Bauer disc diffusion method on Mueller-Hinton agar (Clinical and Laboratory Standards Institute, 2016). A sterile cotton swab stick was dipped into the adjusted suspension. The surface of the sterile agar was inoculated by streaking the swab over the entire sterile agar surface. The inoculated plates were impregnated with discs such as: penicillin G (10µg), Augmentin (30µg), Streptomycin (10µg), Ciprofloxacin (5µg), Nalidixic acid (30µg), Gentamycin (10µg), Ofloxacin (5µg), Chloramphenicol (10µg) and so on (Spencer *et al.*, 2014). The plates were inverted and incubated at 37ºC for 24 hours (Clinical and Laboratory Standards Institute, 2014). Zones of inhibition were measured to the nearest whole millimeter using a meter rule after 24 hours of incubation (Kolo, 2016).

Interpretation for Multi drug resistance bacteria

Bacteria isolates resistant to three or more classes of antibiotics according to the clinical laboratory standard institute (CLSI, 2016) guidelines were termed multi-drug resistant (MDR) bacteria (Magiorakos *et al.*, 2012; Iliyasu *et al.*, 2015).

RESULTS

Out of the 390 endocervical swab and the 390 urine samples screened from 390 patients, only 240(62%) samples revealed the presence of various bacteria (Table 1). Specifically, 28.2% of the 390 ESC samples and 33.3% of the 390 urine samples were positive for various bacterial isolates (Table 1).

Table 1: Prevalence of bacterial infection in PID patients attending three general hospitals

Samples	Number of Samples Screened	Number of Samples Screened	Prevalence (%)
Endocervical swab	390	110	28.2
Urine	390	130	33.3
Total		240	62

Table 2 shows that out of 240(62%) samples positive for various bacterial isolates, 23(20.9%) and 27(20.8%) bacterial isolates in ECS and urine samples were positive for *S. typhi*.

Table 2a: Occurrence of *Salmonella typhi* in three general hospitals

Bacterial isolates	ECS Number (%)	URINE Number (%)	Total Number (%)
Salmonella typhi	23(20.9)	27(20.8)	50(20.8)
Total	110	130	240

KEY: ECS= Endo cervical swap

Table 2b: Number of samples positive for *Salmonella typhi* in each general hospital

Hospitals	ECS Number (%)	URINE Number (%)	TOTAL Number (%)
G.H.Bida (n=130)	8(34.8)	9(33.3)	17(34.0)
G.H.Suleja (n=130)	6(26.1)	8(29.6)	14(28.0)
G.H.Kontagora (n=130)	9(39.1)	10(37.0)	19(38.0)
Total	23(100)	27(100)	50(100)

KEY: n= Specific number of samples collected from each hospital; ECS= Endo cervical swap; G.H= General Hospital

Table 3 shows that out of the 50(20.8%) *S. typhi* isolates there were 18(36%) multidrug resistant *Salmonella typhi* with 10(37%) from urine and 8(34.8%) from endocervical samples.

Table 3a: Multi drug resistant (MDR) *Salmonella typhi* in patients with PID from three general Hospitals

Bacterial isolates	ECS Number (%)	URINE Number (%)	TOTAL Number (%)
MDR - *Salmonella* Typhi	8(34.8)	10(37.0)	18(36.0)
Total	23	27	50

KEY:
ECS= Endo cervical swab, MDR= Multidrug resistant

Table 3b: Number of multi drug resistant (MDR) *Salmonella typhi* in patients with PID in each general hospital

Hospitals	ECS Number (%)	URINE Number (%)	TOTAL Number (%)
G.H.Bida	4(50.0)	5(50.0)	9(50.0)
G.H.Suleja	2(25.0)	2(20.0)	4(22.2)
G.H.Kontagora	2(25.0)	3(30.0)	5(27.8)
Total	8(100)	10(100)	18(100)

KEY: ECS= Endo cervical swap; G.H= General Hospital

Salmonella typhi from General hospital Suleja and General hospital Kontagora showed highest resistance (100%) to Ofloxacin, Streptomycin, Cephalexin, Nalidixic acid, Perfloxacin and Augmentin. However, 66.7% and 80% of *Salmonella typhi* were susceptible to Ciprofloxacin and Ampicillin in General hospital Bida and General hospital Kontagora respectively.

Table 4: Susceptibility Pattern of multidrug resistant *Salmonella typhi* in patients with PID from three general hospitals

Hospitals	No of Isolates	Susceptibility pattern	OFX(%)	PEF(%)	CPX(%)	AU(%)	CN(%)	ST(%)	CEP(%)	NA(%)	SXT(%)	PN(%)
G.H.S	4	S	0 (0)	2 (50)	2 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
		I	0 (0)	1 (25)	1 (25)	2 (50)	1 (25)	0 (0)	0 (0)	0 (0)	1 (25)	1 (25)
		R	4 (100)	1 (25)	1 (25)	2 (50)	3 (75)	4 (100)	4 (100)	4 (100)	3 (75)	3 (75)
G.H.B	9	S	4 (44.4)	4 (44.4)	6 (66.7)	2 (22.2)	2 (22.2)	2 (22.2)	1 (11.1)	1 (11.1)	4 (44.4)	1 (11.1)
		I	1 (11.1)	4 (44.4)	2 (22.2)	3 (33.3)	1 (11.1)	3 (33.3)	3 (33.3)	1 (11.1)	3 (33.3)	1 (11.1)
		R	4 (44.4)	1 (11.1)	1 (11.1)	4 (44.4)	6 (66.7)	4 (44.4)	5 (55.6)	7 (77.8)	2 (22.2)	7 (77.8)
G.H.KN	5	S	2 (40)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	3 (60)	2 (40)	1 (20)	4 (80)
		I	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)	2 (40)	1 (20)	2 (40)	1 (20)	0 (0)
		R	3 (60)	5 (100)	4 (80)	5 (100)	4 (80)	3 (60)	1 (20)	1 (20)	3 (60)	1 (20)

Key:OFX:Ofloxacin;PEF:Perfloxacin;CPX:Ciprofloxacin;AU:Augmentin;CN:Gentamicin;ST:Streptomycin;Cep:Cephalexin;NA:Nalidixicacid; SXT:Sulfamethoxazole; PN:Ampicillin; S: Susceptible; I:Intermidiate; R:Resistance; G.H.S: General Hospital Suleja; G.H.B: General Hospital Bida; G.H.KN: GeneralHospitalKontagora

DISCUSSION

The result from this study, indicates that 240(62%) of the samples collected from PID patients were positive for various bacterial growth. This is based on the silent spread of bacteria organisms to the upper genital tract which results to high degree of damages such as miscarriage, preterm labor and ectopic pregnancy in the infected females (Naaz *et al.*, 2016). These therefore lead to infertility among the female population (Oseni and Odewale, 2017; Ahmed 2017). This is in agreement with the findings of (Pachori and Kulkarni, 2016; Naaz *et al.*, 2016) who reported that higher rates of bacterial infections such as 60%, 57% and 30% in Africa, Asia and Indian respectively.

The high occurrence of *Salmonella typhi* 50(20.8%) revealed in this study, could be based on the fact that *Salmonella typhi* is one of the intracellular pathogens that is predominantly associated with gastroenteritis, which is accompanied with frequently diarrheoa and as such exposes this organism to the vagina due to its proximity to the periurethral openings and the perianal areas. This results is in agreement with the findings of Erdem *et al.*, 2018, who revealed that majority of the organisms isolated from patients with urogenital infections are mainly enteric bacteria such as *E.coli* and *K.pneumoniae*. Similarly, the high occurrence of *Salmonella typhi* observed in General Hospital Kontagora 19(38.0%) could be based on the fact that there is a high rate of bacterial infection especially, *Salmonella typhi* infection among residents in Kontagora local government area (L.G.A).

Furthermore, the high occurrence of multidrug resistant-*Salmonella typhi* 18(36.0%) revealed in this study, could be based on the fact that due to selective toxicity few species of *Salmonella typhi* are constantly exposed to overused antibiotics, hence the development of few multidrug resistant organisms. This result disagrees to the study of Metri *et al.*, 2012, who revealed that 58 bacteria isolates associated with urogenital infections, were multidrug resistant *Klebsiella pneumoniae*. Similarly, the high rate of multi drug resistance in *Salmonella typhi* isolated from General Hospital Bida 9(50%) could be based on the fact that most patients whose bacterial infections are caused by *Salmonella typhi*, practiced indiscriminate use of antibiotics, thus enhancing the development of multi drug resistance in *Salmonella typhi*.

Highest resistance (100%) exhibited by multidrug resistant-*Salmonella typhi* to Ofloxacin, Perfloxacin, Augmentin, Cephalexin, Nalixidic acid and Streptomycin in this study, could be attributed to the fact that most of these bacterial isolates exhibit high horizontal gene transfer; which is the basis of multidrug resistance among bacterial isolates. In addition to this, these isolated multidrug resistant organisms are involved in the rapid dissemination of resistant genes among the intra and inter species in the study area, and as such, infection of the pelvic region by these resistant organisms among the reproductive age women, have led to prolonged hospital stay, high morbidity and high mortality (Oyedum *et al.*, 2021; Usman 2016). This result agrees with the findings of Iseghohi, 2016, who reported that high multidrug resistant isolates were resistant to commonly prescribed antibiotics.

CONCLUSION AND RECOMMENDATIONS

This study revealed that 240(62%) of the samples from General Hospitals Suleja, Bida and Kontagora were positive for bacterial infection. However, 50(20.8%) of these bacterial isolates were confirmed to be *S. typhi*. This study also revealed that 18(36.0%) multidrug resistant *Salmonella typhi* were associated with pelvic inflammatory disease (PID) and as such, these multidrug resistant *Salmonella typhi* revealed highest resistance (100%) to six (6) antibiotics in the three General Hospitals studied. Therefore, it is necessary that the government enforce certain measures such as; constant awareness on the misuse of antibiotics and laws against self-medication and illegal purchase of drugs across the counter to help curb the menace associated with pelvic inflammatory disease that have resulted from resistant organisms.

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