GENETIC PROFILING OF PATHOGENS ASSOCIATED WITH PYROXIA OF UNKNOWN ORIGIN TREATED FOR PLASMODIASIS IN BAYELSA STATE.

ABSTRACT

Antimicrobial resistance of *Salmonella* is an increasing problem and has become a public health issue worldwide. Moreover the evidences on hazard of therapeutic failure due to the increasing incidence of antimicrobial resistance among typhoidal antibodies and malaria are on the increase. The study was aimed at the genetic profiling of pathogens associated with pyroxia of unknown origin treated for Plasmodiasis in Bayelsa State. A total of 1200 samples of 200 Stool and 1000 blood were collected from consented subjects in Federal Medical Center and Niger Delta University Teaching Hospital Bayelsa State. The blood samples were used for malaria parasites and Typhoidal antibodies while the stool samples were used for culturing on Salmonella-Shigella agar. Antibiotic sensitivity of the pure isolates were carried out using sensitivity disc, biochemical analysis using API 20E was carried out to actually differentiate bacterial from each isolates and finally by genetic profiling of the resistant genes of the pure isolates using polymerase chain reaction. Antibiogram of the isolates showed high resistance to oxacillin (73.6 %), ampicillin (66.6%), Tetracycline (60.0%), and Erythromycin (53.3%) while Gentamycin, 22(73.3%), Chloramphenicol 18(60%), and Azithromycin 18(60%) had the highest sensitivity. The distribution of resistant genes from the genetic profiling showed that out of 20 amplified isolates, NDM resistant genes were 17(85%) followed by Bla TEM genes 14(70%) followed by CTXM and SHV genes as 11(55%) and 10(50%) respectively. The bacterial isolates showed high AMR and MDR to the commonly used antimicrobial drugs and the distribution of ESBL genes revealed a high level of resistant genes in Bayelsa State.

Keywords: ESBL, MDR, AMR, Malaria, Fever

INTRODUCTION

Pyrexia of unknown origin have been a recurrent issue in Nigeria where malaria is endemic and antibiotic-resistant bacteria are a major threat to public health, causing community outbreaks of infectious diseases. Moreover the evidences on hazard of therapeutic failure due to the increasing incidence of antimicrobial resistance among typhoidal antibodies and malaria are on the increase (4). Also, the antimicrobial resistance of *Salmonella* is an increasing problem and has become a public health issue worldwide (14). Antibiotics with the greatest percentage of resistant isolates include Amoxicillin, Clavulanic acid, Ampicillin, Ceftiofur, Cefoxitin, Chloremphenicol, Streptomycin, Sulfonamides, and Tetracyclines; however, the percentage of isolates resistant to these drugs has increased since 1997.

Many patients engage in antibiotic self-medication, which limits the number of positive cultures which are reported and also are major causes of antibiotics resistant in the treatment of typhoid fever (6).

Apart from being costly, the culture facilities are limited outside the teaching hospital and they are not employed on a routine basis. Moreover, the isolation and the identification of

organism may take several days (18). Several methods have been developed for the detection, identification and characterization of resistant genes of *Salmonella* species. Some of thse methods include, the amplification of 16s RNA, multiplex sequencing typing and other methods (20).

Molecular diagnosis of bacteria is one of the preferred method and most accurate method in the diagnosis but may be very expensive and not accessible in our rural areas for effective

diagnosis (20). Culture can take from 4 to 7 days in order to isolate and confirm the presence of bacteria from the sample (10). Conventional culture methods used for the isolation of *Salmonella* include, non-selective pre-enrichment followed by selective enrichment and plating on selective and differential agars. Suspected colonies are then confirmed biochemically and serologically. More recently, a number of alternative methods for the detection of bacteria in stool have been developed including, immune-assays, nucleic acid hybridization and polymerase chain reaction (PCR) techniques (23). The Polymerase Chain Reaction (PCR) has become a powerful tool in microbiological diagnostics during the last decade. PCR based methods combine 4 simplicity with a potential for high specificity and sensitivity in detection of pathogens.

AIM

Genetic profiling of pathogens associated with pyroxia of unknown origin treated for Plasmodiasis in Bayelsa State.

MATERIALS AND METHODS

This study was carried out at Federal Medical Center Bayelsa State and Niger Delta University Laboratory Amassoma in Bayelsa State. This study was carried out for duration of one year. Consented subjects were enrolled for this simple random sampling method.

Stool and blood samples of febrile patients associated with Typhoidal antibodies and malaria parasites from male and female were all collected for the study.

SAMPLE COLLECTION

Stool and blood samples were collected from consented subjects in Federal Medical Center and Niger Delta University Teaching Hospital Bayelsa State. A total of 1200 participants

were recruited for this study. The blood samples were used for testing for malaria parasites while the stool samples was used for culturing on Salmonella-Shigella agar. Antibiotic sensitivity of the pure isolates were carried out using sensitivity disc, biochemical analysis using API 20E was carried out to actually differentiate bacterial from each isolates and finally by genetic profiling of the resistant genes of the pure isolates using polymerase chain reaction

Ethical Clearance

An informed, written consent was obtained from all participants after explanation of the purpose of the study. Ethical approval was also obtained from the ethics committee from Federal Medical Center Bayelsa State where the study was carried out.

RESULTS

The demonstration of antiobotic susceptibility testing of the studied isolates showed that chloramphenicol had the least resistant of (0)0.0% with ampicillin showing the highest resistant value of 20(66.6%).

Furthermore, Gentamycin had the highest susceptibility of 22(73.3%) followed by Chloramphenicol and Azithromycin 18(60%) each. while Erythromycin 4(13.3%).

More so, all the antibiotics reported varying intermediate values which ranges according to the measurements of clarity on plates (Table 1)

Table 1. Susceptibility of the studied Isolates

	Resistant		N = 30	Susceptible	
Antibiotics	F	%	F	%	

Ampicillin	20	66.6	5	16.6
Azithromycin	5	16.6	18	60.0
Ciprofloxacin	2	6.6	10	33.3
Ceftriaxone	5	16.6	16	53.3
Erythromycin	16	53.3	4	13.3
Gentamicin	4	13.3	22	73.3
Rifampicin	7	23.3	13	43.3
Choramphenical	0	0.0	18	60.0
Augumentine	4	13.3	17	56.6
Streptomysin	8	26.6	5	16.6
Total				

KEY:

F= Frequency

N= Number of isolates

The study revealed that out of the 200 culture isolates, 33 (16.5%) produced hydrogen sulphides when exposed to API20E.

Our findings further revealed that of the 33 isolates that produced hydrogen sulphide, 29(87.8) were urease positive while 4(12.1%) were urease negative when exposed to API technique (Table 2).

Table 2 Number and Percentage of Stool Samples Isolates exposed to API20E

RESULT		**(n=200)**
	H_2S (%)	**Urea(%)**
Positive	33(16.5)	29(87.8)
Negative	167(83.5)	4(12.1%)
Total	200 (100)	33(100)

The distribution of resistant genes among the bacterial isolates demonstrated that out of 20 bacterial isolates, 1(5%) had the NDM genes.

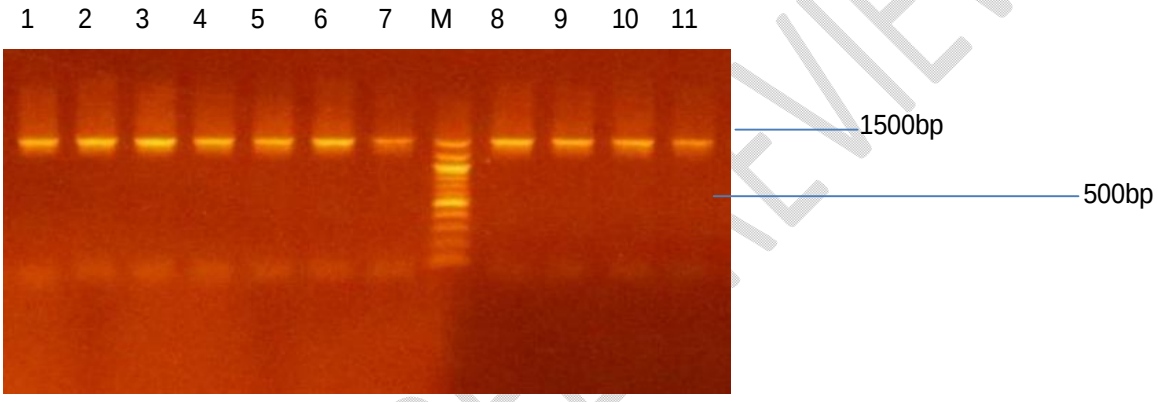
Our findings further revealed that the bacterial isolates had 14(70%), 17(85%) and 10(50%) for BlaTem, CTXM and SH respectively (Table 3).

Table. 3 The Distribution of Resistant Genes among the selected Bacterial Isolates

Genes	**Total No. of Isolates**	**No. of Resistant Isolates**
BlaTem	20	14(70%)
CTXM	20	17(85%)
NDM	20	1(5%)
SHV	20	10(50%)

The agarose gel plate 16srRNA PCR amplification showed the 16srRNA gene was 1500 base pair on the plate. The 1500bp represents the molecular weight of the gene. The molecular weight of each amplified gene is determined by subjecting the amplicon on gel electrophoresis at 130 volt, 500 milliAmpire within 25 -30 minutes for migration of bands according to their sizes alongside with the DNA ladder (Plate 1).

PLATE 1: 16SrRNA PLATE

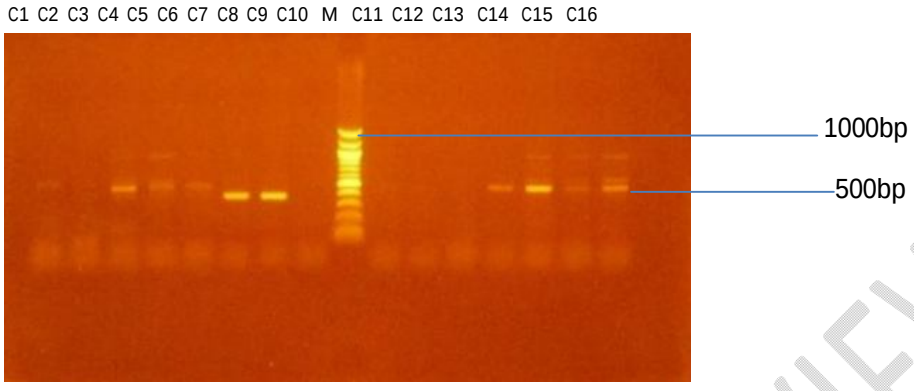


Agarose gel electrophoresis of some selected bacterial isolates. Lane 1 – 11 represents 16SrRNA gene bands (1500bp). Lane M represents the 100bp Molecular ladder.

Agarose gel electrophoresis of CTX-M gene of some selected bacteria isolates. Lane C1, C2, C3, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14 C15 and C16 represent the CTX-M gene bands (550bp). Lane M represents the 100bp Molecular ladder.

The agarose gel plate CTX-M PCR amplification, showed that CTX-M gene was 550 base pair on the plate and the 550bp represents the molecular weight of the gene (Plate 2).

PLATE 2: CTXM PLATE



Agarose gel electrophoresis of CTX-M gene of some selected bacteria isolates. Lane C1, C3-C5 and C12-C16 represents the CTX-M gene bands (500bp). Lane M represents the 100bp Molecular ladder.

Agarose gel electrophoresis of NDM gene of some selected bacteria isolates. Lane C1 C2 C3 C4 C5 C6 C7 C8 C9 C10 C11 C12 C13 C14 and C15 represent the NDM gene band (621bp). Lane M represents the 100bp Molecular ladder of 1500bp.

The agarose gel plate of NDM PCR amplification showed the NDM gene was 621 base pair on the plate which represents the molecular weight of the gene (Plate 3).

PLATE 3: NDM PLATE

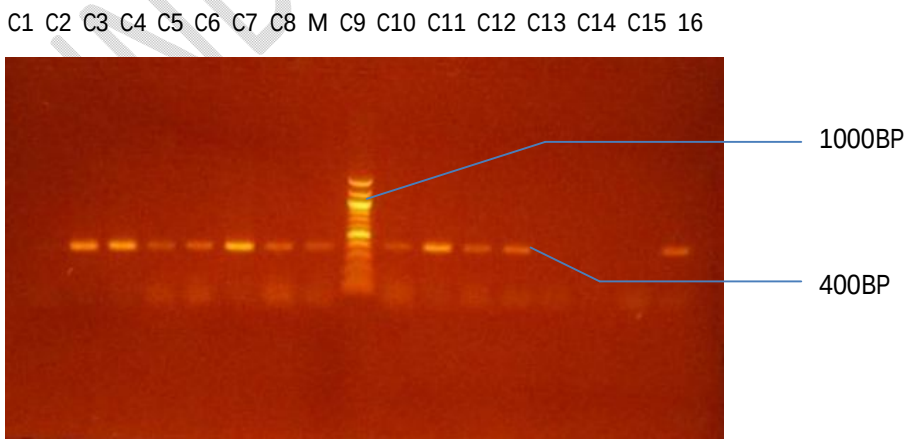
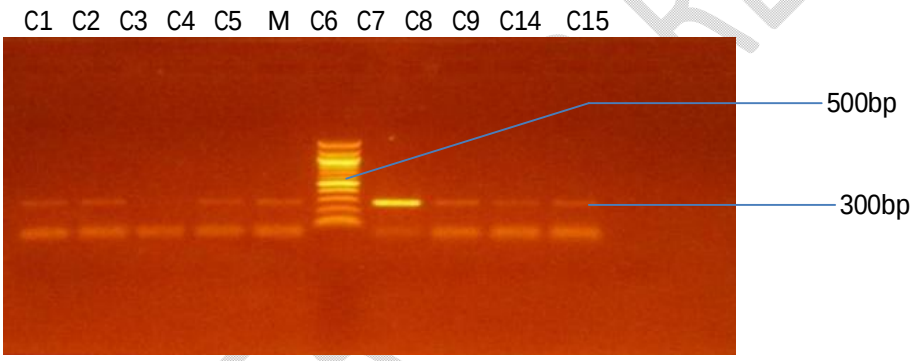


PLATE: Showing Agarose gel electrophoresis of BlaTEM gene of some selected bacterial isolates. Lane C2 – C12, and 15 represents the BlaTEM gene bands (400bp). Lane M represents the 100bp Molecular ladder of

Agarose gel electrophoresis of SHV gene of some selected bacteria isolates. Lane C1, C2, C3, C4, C5, C6, C7, C8, C9, and C15 represent the SHV gene bands (293bp). Lane M represents the 100bp Molecular ladder.

The agarose gel plate of SHV PCR amplification revealed the SHV gene is 281 basepair on the plate which represents the molecular weight of the gene (Plate 4).

PLATE 4: SHV PLATE



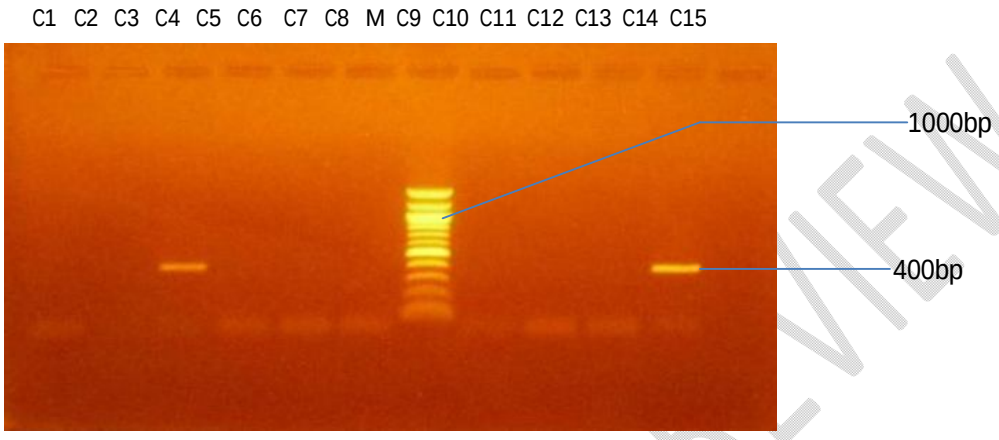
Agarose gel electrophoresis of some selected bacterial isolates. Lane C1 – C5, C6 – C14 represents SHV gene bands (300bp). Lane M represents the 100bp Molecular ladder.

Agarose gel electrophoresis of BlaTEM gene of some selected bacteria isolates. Lane C1, C2, C3, C4 C5, C6, C7, C8, C9, C10, C11, C12, C13, C14 and C15 represent the BlaTEM gene bands (401bp). Lane M represents the 100bp Molecular ladder

The agarose gel BlaTEM PCR amplification showed the BlaTEM gene is 400 basepair on the plate which signifies the molecular weight of the gene.

(Plate 5)

PLATE 5: BlaTEM PLATE



Agarose gel electrophoresis of SHV gene of some selected bacteria isolates. Lanes C1 – C8 and C10 – C14 represent a TEM band (400bp). Lane M represents the 100bp Molecular ladder.

DISCUSSION

Antibiotics resistant have been established in this study to be a serious concern from febrile patients in Bayelsa State.

After culturing faecal samples on DCA and SSA culture, it was further subjected to API test, which 4(12.1%) were negative to Urease and 29(87.8%) were positive to urease a pointer of co-infection (*Salmonennae Proteus* infection) or high sensitivity of the API kit.

The isolate resistant to a range of antimicrobials have emerged and threaten to become a severe public health problem (24). This resistance results from the misuse of antimicrobials. In this study, 30 isolates were examined against 10 different antimicrobial agents belonged to various groups such as quinolone, phenicol, βlactams, aminoglycosides, tetracyclines, cephalosporins, and sulfonamide groups.

Antibiogram of the isolates showed high resistance to ampicillin (66.6%) and Erythromycin (53.3%). The obtained result was similar with Habrun *et al.*, (2012), who reported that 100%

of the isolates were sensitive to Chloramphenicol and streptomycin, while 92 of the isolates (58%) were susceptible to nalidixic acid. In addition, Okamoto *et al*., (2009) reported that Chlorophenicol is the most effective antimicrobial for treating Salmonella infection.

Chloramphenicol, which other antibiotics were compared with, has been the "gold standard" therapy since its introduction in 1948. Treatment with Chloramphenicol reduces typhoid fever mortality from approximately 20% to 1% and fever from 14-28 days to 3-5 days (15). The reemergence of certain bacterial isolates to Chloramphenicol and study results in India showed that a high sensitivity of *Salmonella* enterica serovar Typhi to Chloramphenicol (96%) (7).

Contrary to this present study, a high resistance rate of bacteria agents to ceftriaxone (75%), Chloramphenicol (83.7%), and gentamicin (75.6%) have been reported Asrat (2018). Also, Cardoso *et al*. (2006) said that 100% of Salmonella isolates showed sensitivity to doxycycline, while, Shivhare *et al*., (2000) reported high sensitivity of *Salmonella* spp. to ofloxacin, while all the isolates were resistant to sulfonamides trimethoprim. Moreover, our results agreed with Snow *et al.,* (2007), who reported that all isolates from commercial layer flocks in the UK were sensitive to amikacin. The products in this study are similar to Zdragas *et al.,* (2012), who reported 5% resistance to streptomycin and 2% to nalidixic acid but differed from Khan *et al.* (2010) 87.9% of *Salmonellae* were sensitive to ciprofloxacin. However, in this study, an intermediate resistance was detected in *S. typhi* isolate for ciprofloxacin. This finding is of concern as ciprofloxacin is commonly used to treat other bacterial infections in the study area. It is also used to treat enteric fever due to the emergence of multiple drug resistant (MDR) to first-line drugs like Chloramphenicol, ampicillin, and cotrimoxazole.. All isolates were resistant to more than one antimicrobial, indicating multiple drug resistance. These findings confirm that humans are reservoirs of multi-resistant *Salmonella*. However, this did not correlate with the antimicrobial resistance gene, suggesting that other mechanisms of acquired resistance could be present (2, 19).

The agarose gel electrophoresis plate in this study showed 16srRNA PCR amplification. The 16srRNA gene is 1500 basepair.

Various virulence determinants in *Salmonella species* are associated with chromosomal and plasmid factors (19). All the identified isolates were subjected to PCR genotyping for the detection of some virulence determinants.

Molecular identification of the bacterial isolate resistance genes showed that the blaTEM gene, a gene encoding for β-lactamases resistance, was reported in the present study. This study result showed that 14(70%) out of 20 bacterial isolates had the blaTem gene which was similar to an analysis performed by Hur *et al.,* (2011), who said that 90.5% of penicillin-resistant bacterial isolates carried the blaTEM gene. In another study, the percentage of blaTEM was 10% which was identified in ten bacterial isolates (Ahmed *et al.* (2009). However, the blaTEM gene was detected in 51.6% resistant bacterial isolates from the study of Yang *et al.,* (2010). Aslam *et al*. (2012) reported that the percentage of blaTEM gene in isolated from retail meats in Canada was 17%, and this gene was the most common resistance gene found. According to Lu (2011), a total of 108 *S. indiana* possessed the 81.2% blaTEM gene in their study (Lu, 2011). Similarly, SHV gene, a gene encoding for streptomycin resistance, was reported in the present study with a rate of 10(50%). These results agree with an analysis performed by Shahada *et al.* (2006), who noted that all streptomycin-resistant *S. infantis* in Japan carried the SHV gene, while Mohamed (2004) recorded 53.1% of study isolates possessed the SHV gene whereas on the contrary, Sheng *et al*. (2004) reported a lower rate of SHV gene (3%) in only three isolates out of seventy-three bacterial isolates.

The results of this current study also revealed the detection of 16srRNA and NDM genes in the examined isolates. The detection of the 16srRNA NDM gene in the isolates was 17(85%) out of 20 bacterial isolates which was also reported in other studies (Borges *et al.,* 2013; Ahmed, 2016). However, lower frequencies of 80% (Streckel *et al*., 2004) and 50% in Zou *et al.* (2011) were reported in bacteria isolates. This variation could be attributed to recombination, which frequently occurs in the location of this gene (Hopkins & Threlfall, 2011). Consistent with the current results of the 16srRNA gene, it was previously identified in 100 and 88.2% of isolated from chicken samples in Brazil (Boerges *et al.,* 2013) and Egypt (Ammar *et al*., 2016), respectively.

Regarding results of the NDM gene, the obtained frequency of the NDM gene was comparable with other findings (Chuanchuen *et al.,* 2010; Ahmed, 2016). However, different studies in Egypt reported detecting the gene with higher frequencies ranging from 100% (Gharieb *et al.,* 2015) to 41.2% (Ammar *et al.,* 2016). The considerable differences in virulence determinants of *Salmonella* serovars are attributed to the variation in sample sources, types of serovars, and the presence or absence of plasmids carrying virulence-associated genes (Porwollik *et al.,* 2004).

These results indicate a high probability of identity between the R-plasmids found in bacterial, which usually encoded resistance to amoxicillin, Tetracycline, Chloramphenicol, sulfamethoxazole-trimethoprim, and cotrimoxazole. The cause of the increase in R factor-carrying bacteria is the selective pressure caused by antibiotics. It has been shown that the use of antibiotics in animals significantly increases the pool of R factor-carrying bacteria in the environment. It seems likely that the use of antibiotics for other non-medical purposes also helps the increase of the reservoir of R factors (Hussian, 2015). Many antimicrobial agents include penicillin, cephalosporin, Tetracycline, spectinomycin, Chloramphenicol, fusidic acid, sulfonamides, heavy metal, and other plasmid-mediated antibiotic resistance has been reported (3, 8).

Conclusions

Drug misuse and unprescribed medication is a great challenge to the world as more bacteria have continue to produce antibiotic gene resistant. The high susceptibility of chloramphenicol, gentamycin and ceftriaxone are indicative of the sustenance in the use of gentamycin and ceftriaxone as first-line antibiotic options for typhoid fever. The bacterial isolates showed high AMR and MDR to the commonly used antimicrobial drugs and the distribution of ESBL genes revealed a high level of resistant genes in Bayelsa State.

It could be concluded that the antimicrobial resistance genes of the bacterial isolates were extensively vast in the study area, thus leading to minimizing the influence of antibiotics efficacy in both treatment and prevention. Therefore, other tools of prevention and treatment are essential to avoid this problem.

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