Mutations in the Quinolones Resistance Determining Regions of gyrA in Nosocomial Staphylococcus aureus and Salmonella typhi

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Abstract

In Nigeria, Staphylococcus aureus and Salmonella typhi are common causes of human infections and are also recognized as pathogens of public health significance. This study therefore, sought to determine the incidence and extent of fluoroquinolones resistance of S. typhi and S. aureus isolated from patients in Nigerian Defence Academy Hospital. A total of 60 samples obtained from patients with request for stool microscopy, culture and sensitivity, wound swabs, indoor air of surgical wards and swabbing of working benches were analyzed for the presence of S. aureus and S. typhi. The bacterial isolates were then subjected to antibiotic sensitivity testing using a modified Kirby-Bauer disk diffusion method. The antibiotic susceptibility patterns of S. typhi revealed that some of the isolates were resistant to two or more fluoroquinolones namely: ciprofloxacin, sparfloxacin, ofloxacin and pefloxacin. S. aureus on the other hand, also showed resistance to fluoroquinolones. The isolates that showed resistance to more fluoroquinolones were taken for molecular analysis. The genomic DNA was extracted and amplified using specific primer for gyrA by PCR, visualized using agarose gel electrophoresis and then sequenced. The amplicon sizes were 251bp respectively for each of the isolates. The detection of resistant pattern responsible for fluoroquinolones resistance showed that mutation had occurred. Mutation in nucleotide sequence was detected in gyrA gene of the fluoroquinolone resistant strains.

Keywords: Agarose Gel Electrophoresis, Fluoroquinolones, gyrA Gene, Mutation, S. aureus, S. typhi.

Introduction

Health care facilities such as hospitals, rehabilitation units and outpatient departments are places where people seek attention from their medical problems. Nosocomial infection is a global problem, affecting 6% of hospitalized patients and in some clinical services such as intensive care units, up to 5% of the patients are infected (Madigan et al., 2000). Over two million nosocomial infections are usually recorded each year worldwide and only few organisms
cause majority of the infections at several sites (Lark, 2001). The most frequent infections are from surgical wounds, blood, urinary and gastro-intestinal tract infections. Factors that enhance the spread of nosocomial infections include weak immunity and the increasing variety of medical and surgical routes of infection (for example, patients with open wounds or tube in their body) as well as transmission of drug-resistant micro-organisms among crowded hospital populations where poor infection control practices exist (Amita et al., 2003). Reports have implicated Staphylococcus aureus, Escherichia coli and Salmonella typhi as the causative agents of hospital acquired infections (Lark, 2001). These organisms can grow at different temperatures and pH conditions in the hospital environment, and in addition, are able to exploit varieties of carbon and energy sources. These characteristics explain the ability of these pathogens to survive for a reasonable time in either dry or moist conditions in the hospital environment, causing infection (Beaudin et al., 2004). Salmonella typhi on the other hand, causes typhoid fever (Mirza et al., 2006). The human specific pathogen has evolved some mechanisms for persistence in its host that ensure its survival and transmission (Parry et al., 2005). The global estimates for typhoid fever are 2.1 million episodes annually with 216 000 deaths (Crump et al., 2004).

Human beings are the carriers of S. typhi and S. paratyphi without any environmental reservoirs (Mirza et al., 2006). In third world countries, great reliance is placed on antibiotic chemotherapy in the treatment of typhoid fever because of the difficulties in preventing typhoid fever due to inadequate public health awareness.

Efforts to control diseases caused by nosocomial S. aureus and S. typhi using antibiotics have resulted in increased resistant strains of these organisms (Levy, 1998). Therefore, in order to effectively treat infections caused by these two pathogens, culture and antibiotic sensitivity tests should first be conducted. Once culture and sensitivity results confirm bacterial infection and sensitivity pattern, treatment may be modified (Paterson, 2000).

Materials and Methods

Sampling Technique

A multi-staged sampling technique was employed for isolation of S. typhi while S. aureus was isolated from wound swabs, indoor air of surgical wards using pour plate technique and swabbing of working benches and door handles.

Molecular Characterization of Species of Bacteria

The bacterial species that were biochemically characterized were subjected to strain level identification using molecular approach. These involve extracting their DNA using standard protocols, amplifying the DNA using PCR and sequencing the gene of interest using next generation sequencing (Hakanen et al., 2001).

Detection of Mutation in gyrA Gene of the Bacterial Isolates

The genomic DNA of Staphylococcus aureus and Salmonella typhi that was extracted was subjected to PCR searching for gyrA gene. The PCR was operated based on the following conditions: pre-denaturation (94°C for 5 min), 30 cycles of denaturation (94°C for 30 sec), annealing temperature was 48°C for 30 sec, extension was 72°C for 30 sec and final extension was 72°C for 5 min using Perkin Elmer PCR machine. 20 reaction mix tube was used and the reaction volume was 20µl.
The PCR product was ran in 1.5% agarose gel electrophoresis stained with ethidium bromide and viewed under ultraviolet for visible amplified image of the genes. A DNA marker of 100 base pairs was used. The specific primers used to amplify the quinolones resistance determining region (QRDR) were designed from accession number sequences X78977 and M68936 as follows: gyrA- Forward reaction 5’(CGT TGG TGA CGT AAT CGG)-3’ Reverse 5’(CCG TAC CGT CAT AGT TAT) 3’.

Results

PCR Amplification of Genomic DNA (gyrA Gene) from S. typhi and S. aureus that was Resistant to Fluoroquinolone

Agarose gel electrophoresis pattern showing single PCR amplified products of gyrA from S. typhi and S. aureus with resulting amplicon size of 251 base pair bands for both S. typhi and S. aureus. A 100 base pairs molecular marker was used as shown in Figure 1.

Figure 1: Agarose Gel Electrophoresis Pattern showing PCR Amplified Products of gyrA from S. enterica serovar typhi and Staphylococcus aureus.


DNA Sequencing
The PCR products containing the gene of interest were selected for DNA sequencing with the same primer sequence.
The polymerase chain reaction analysis shows that S. enterica serovar typhi and S. aureus possessed mutation in the gyrA gene. The outcome of the DNA sequence of the PCR product of S. typhi and S. aureus gyrA gene was given as shown below (Figure 2 & 3):

Salmonella typhi
TCAGTTTCAATCGATGCAATTTGCGCCAGA CGGATTTCCGTATAACGCGATTGCGCC GAGAGTCGCCGTGATGGAACCGAA GTTACCCCGGCCCTCTACCAGCATGT

S. aureus
TCAGTTTCAATCGATGCAATTTGCGCCAGA CGGATTTCCGTATAACGCGATTGCGCC GAGAGTCGCCGTGATGGAACCGAA GTTACCCCGGCCCTCTACCAGCATGT
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AACCCAGGGGAGGGTGCGCCAT  
ACTGACTATCTGCTCATAACCCGCAA.  
The outcome of the DNA sequence was blasted using the National Centre for Biotechnology Information database and the result was as follows:  
Sequence ID: lcl|Query_32315  
Length: 563  
Number of Matches: 1  
Related Information

Range 1: 239 to 392 [Graphics] Next Match Previous Match

Alignment statistics for match #1

<table>
<thead>
<tr>
<th>Score</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>200 bits(108)</td>
<td>3e-56</td>
<td>139/154(90%)</td>
<td>2/154(1%)</td>
<td>Plus/Minus</td>
</tr>
</tbody>
</table>

Query 1

TCAGTTTCATGGGCAATTTTCGCCAGACGGATTTCCGTATAACGCATTGC
GATC

\[\text{Sbjct 392} \]

TCAGTTTCATGGGCAATTTTCGCCAGACGGATTTCCGTATAACGCATTGC
GATC

Query 59

CGCCGTCGATGGAAACCGAAGTTACCCCAGGCCCTCTTACCAGCATGTAAACCCAGGGGG
AAAGG

\[\text{Sbjct 332} \]

CGCCGTCGATGGAAACCGAAGTTACCCCAGGCCCTCTTACCAGCATGTAAACCCAGGGGG
AAAGG

Query 119

GTGGCGCCATACTGACTATCCTGTCATAAACCCGC

\[\text{Sbjct 272} \]

GTGGCGCCATACTGACTATCCTGTCATAAACCCGC

Figure 2: Blast Sequence showing Mutation in *Salmonella typhi.*

From the above, there was removal of two bases in query one meaning that deletion mutation has taken place, and in query 59 and 119 there was exchange of single nucleotide for another meaning that substitution mutation has occurred as shown in Figure 2.

*Staphylococcus aureus*  

TATAACGCATTGTGTGCCCATCTCCATCCCATTTGAACCAAAGTGCTTGGCCATCAA
CAAGCGATAACGTATACTGAATC
TTGAGCCATACGGATTGCTTCCATAATAGATGATGTCACCATGAGGTGATATTTA
CCGATTACGTACCAACG.

The result of the DNA sequence of the PCR product of *S. aureus* gyrA gene was given as shown below (Figure 3):

The outcome of the DNA sequence of the PCR product of *S. aureus* gyrA gene was given as shown below (Figure 3):

The result of the DNA sequence was blasted using the National Centre for Biotechnology Information database and the result was as follows:
Staphylococcus aureus strain N21OS gyrase
subunit A (gyrA) gene, partial cds
Sequence ID: KX819742.1Length: 492Number of Matches: 1
Related Information
Range 1: 210 to 370GenBank Graphics Next Match Previous Match
Alignment statistics for match #1
Score Expect Identities Gaps Strand
254 bits(137) 5e-64 154/161(96%) 6/161(3%) Plus/Minus
Query 1 TATAACGCATTGCTGCTGC-CCATCTCCATCCATTGAACCAA-GTACCTTG GCCATCAA 57

Sbjct 370 TATAACGCATTGCTGCTGC CCATCTCCATCCATTGAACCAA AGTTACCTTG GCCATCAA

Query 58 CAAGCG-ATAACGATAACTGAA-TCTTGAGCCATACGTACCATTGCTTCATAAATAGATG 115

Sbjct 310 CAAGCGATAACGATAACTGAA ATCTTGAGCCATACGTACCATTGCTTCATAAATAG ATG 251

Query 116 AGTCACCATGAGG-TGATATTTACCGATTACGTCACCAAACG 155

Sbjct 250 AGTCACCATGAGG GTGATATTTACCGATTACGTCACCAAACG 210.

Figure 3: Blast Sequence showing Regions of Mutation in Staphylococcus aureus.

From the above blast sequence, there was removal of three nucleotides in query 1 (deletion mutation), in query 58 there was removal of two nucleotides (deletion mutation) and finally in query 116 there was one exchange of nucleotide for another and removal of one nucleotide (Figure 3).

Discussion

Salmonella typhi isolates were obtained from stool samples and S. aureus from wound swab and swabbing of surgical wards. The presence of S. typhi in stool samples shows a carrier state among the patients. According to Singh (2001), carriers may shed S. typhi either through their stool or urine continuously or intermittently and the carrier state is usually the source of contamination. Carriers can serve as reservoirs or host for the dissemination of S. typhi causing public health problems.

The high yield of S. aureus from swabbing the surgical wards and wound swab is an indication that the wounds serve as a habitat for the organism (Prescott, 2002) and could therefore be one of the major sources of S. aureus infection. S. aureus is a common cause of surgical wound or ulcer infections (Donkor et al., 2008). Most cases of wound infection are acquired either through the hands of a health care worker who is colonized with S. aureus or from the patients’ own reservoir (Sheretz et al., 1996).

The presence of multiple-drug resistant strains of S. typhi and S. aureus among the
isolates may be attributed to two main reasons. Firstly, wrong use of antibiotics arising from self-medication in suspected bacterial infections (Newman et al., 2006). Self-medication prevents early reporting of patients to hospitals at the onset of disease symptoms, except where complications had occurred (Brusch et al., 2010). Secondly, either clonal and/or extrachromosomal resistant genes may be potential mechanisms for the level of multiple-drug resistance as noticed in this study. The implication of the multiple drug resistance recorded in this study is that efficacy of the relatively cheap empirical therapy for S. typhi and S. aureus infections could be jeopardized.

There have been reports from different parts of the world about resistance pattern of clinical isolates (Saha et al., 1999). In this study, most of the isolates were found to be resistant to fluoroquinolones namely: Ciprofloxacin, Sparfloxacin, Ofloxacin and Pefloxacin. Some of the isolates were susceptible to Septrin, Levofloxacin and Amoxicillin. Thus, this trend of result supports the work of some authors when they stated that most reports from developing countries are showing MDRST strain and fluoroquinolones resistant strains (Saha et al., 1999).

Many reports have been put forward by several authors (Brusch et al., 2010) that fluoroquinolones are highly effective against susceptible organism, yielding a better cure rate than Cephalosporin. They also stated that unfortunately, resistance to first generation fluoroquinolones is widespread in many parts of Asia (Brusch et al., 2010). Furthermore, they stated that in recent years, third generation Cephalosporins have been used in regions with high fluoroquinolones resistance rates, particularly in South Asia and Vietnam. It has been reported that fluoroquinolone resistance in S. enterica is usually mediated by at least one mutation in a DNA topoisomerase gene. However, in clinical human isolates of Salmonella spp., mutations are usually confined to gyrA. In this study, the mutations detected were found in gyrA genes in the chromosomes of the S.enterica serovars. This trend of results showed that there have been some forms of resistant mutants along the S. enterica serovars’ family, and that the presence of mutations in gyrA gene of the isolates showed that the resistance to fluoroquinolone is evolving in an ominous direction as reported by Brusch et al. (2010).

GyrA mutation is a good marker indicating that fluoroquinolones should not be chosen for treating the respective infection (Randal et al., 2005). This implies that these patients in whom these isolates were recovered from, especially may have been on fluoroquinolone treatment for a long time (Hirose et al., 2002). Also reports have it that S. typhi most commonly develops fluoroquinolone resistance through specific mutations in gyrA and parC, which codes for the binding region of DNA gyrase and topoisomerase IV respectively (Brusch et al., 2010).

It has been stated that transferable resistant to quinolones is sometimes rare in bacteria in vivo, but clonal or resistance due to mutation in chromosomal gene remains the potential mechanisms accounting for high level of reduced fluoroquinolone susceptibility in Southeast Asia (Hakanen et al., 2001). These trends of results or reports are applicable to the analysis obtained in this study, as the mutations found were chromosomally mediated resistant gene.

In this study, the mutations that are responsible for the fluoroquinolone resistance in the gyrA of the Salmonella enterica serovars and Staphylococcus aureus were investigated and the sequences for the Quinolone Resistant Determining Region (QRDR) of the gyrA gene of the isolates which showed resistance to some fluoroquinolones were detected and analysis
revealed mutation. Sequence analysis also revealed that some of the positions of the nucleotides in the gyrA mutation were deleted and some others substituted. This finding is in line with the work of Hirose et al. (2002) when they stated that, a single mutation at either the Ser-83 or the Asp-87 codon were found after sequencing the genes of S. enterica serovar typhi and serovar paratyphi A (Hirose et al., 2002). This indicates that gyrA mutations are of principal importance for the fluoroquinolone resistance of serovars typhi and paratyphi A among the Salmonellae (Hirose et al., 2002).

5. Conclusion
The emergence of antimicrobial resistance in any part of the world may have a global bearing and thus deserves universal attention. Staphylococcus aureus and Salmonella typhi were therefore characterized using conventional methods and confirmatory test. Their antibiotic susceptibility pattern was therefore determined using Kirby-Bauer method and the organisms showed high resistance to fluoroquinolones. Fluoroquinolones resistant trait of the organisms showed that mutation has taken place. However, in this study, it could also be suggested that extensive use and abuse of fluoroquinolone in human diseases could be responsible for the rapidly increasing quinolone resistance of Salmonella enterica and Staphylococcus aureus in this part of Nigeria as observed. Therefore, prescription of these drugs should be done only by medical personnel and appropriate dispensing techniques should be adopted in every hospital to avoid under dosage or over dosage.

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Conflict of Interest
Authors have declared that no competing interests exist.

References


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