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Antimicrobial resistance and molecular characteristics of *N. gonorrhoea* isolates in Ghana

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Abstract

Introduction: Gonorrhoea is a disease associated with humans and it is caused by *N. gonorrhoea*. *N. gonorrhoea’*s ability to evolve and evade various treatment regimens can lead to untreatable Gonorrhoea disease. In the absence of a viable vaccine, national database on antimicrobial resistance (AMR) and molecular characteristics of *N. gonorrhoea,* and the reliance on syndromic management regimes, continuous national antimicrobial resistance surveillance and molecular characterisation of *N. gonorrhoea* remain imperative. Only two gonococcal data describing its molecular characteristics links to AMR have been reported in Ghana.

Secondary *N. gonorrhoea* isolates (n=4) were collected from two metropolises in Ghana: Tamale in the northern sector (n=1), and Accra in the southern sector (n=3). The isolates were characterised using polymerase chain reaction (PCR) targeting the *por*B and *tbp*B genes, and the disk diffusion method was used to evaluate antimicrobial resistance (AMR). NG-MAST and *por*B gene sequence analysis was used to reveal molecular epidemiology and evolutionary trajectory, respectively.

All four isolates showed multidrug resistance to at least four antibiotics. One isolate showed resistance to all seven antibiotics including ciprofloxacin, ceftriaxone, and azithromycin. NG-MAST typing revealed isolates S3 (**MZ313864**) as ST211 while the locus of S2 (**MZ313863**) (*tbp*B) was identified as *tbp*B1844 but not its *por*B locus. Isolate S3 (**MZ313864**) is globally known while S2 (**MZ313863**) is not previously known.

Multidrug-resistance and previously unknown gonococcal variant was recorded. Therefore, continuous AMR and molecular surveillance in Ghana is essential to compliment the syndromic management regime.

Keywords: *Neisseria gonorrhoea*, Antimicrobial resistance, molecular characterisation, Ghana.

Introduction

Neisseria gonorrhoea is a human-associated pathogen that causes gonorrhoea. The World Health Organisation (WHO) estimated 82 million global incidences of gonorrhoea among young adults (WHO, 2020).

Gonorrhoea is associated with maladies such as urethritis, cervicitis, arthritis, endocarditis, infertility, and meningitis among other deleterious conditions when treatment is delayed or unsuccessful (Mayor *et al*., 2012; WHO, 2016a). Other complications such as inflammation of the foetal membrane, infectious miscarriage, premature birth, and early rupture of the amniotic sac have been reported in pregnant women with gonorrhoea infection (Bennett, Dolin, & Blaser, 2014). During vaginal delivery, gonorrhoea is reported to cause blindness in new-borns via vertical transmission (Mayor *et al.*, 2012; WHO, 2016a).

Prior to antimicrobials, several treatment of gonorrhoea included crude and painful techniques such as genital hyperthermia (Unemo & Shafer, 2014). However, the use of antimicrobials in treatment of gonorrhoea has been successful in the absence of viable vaccine, until the emergence of multidrug resistant strains of *N. gonorrhoea* (Hook & Kirkcaldy, 2018; Unemo & Shafer, 2014). This development has made multidrug-resistant *N. gonorrhoea* a global health concern (Unemo & Shafer, 2014).

The ability of *N. gonorrhoea* to resist antimicrobials differs in various parts of the world and at different times (Hill *et al.,* 2016; Newman *et al.,* 2015; WHO, 2016b, 2018). The lack of effective diagnostic tools and the over-reliance on symptomatic diagnosis and treatment in lower-income countries especially in Africa may contribute to the widespread emergence of resistant gonococcal strains (Unemo & Shafer, 2014; WHO, 2016b).

N. gonorrhoea Multi-Antigen Sequence Typing (NG-MAST), a widely used epidemiological online platform currently hosted by www.pubmlst.org as ng-mast v2.0 has been employed in the identification of clusters of gonococcal strains with the same characteristics (Martin, Ison, Aanensen, Fenton, & Spratt, 2004). Furthermore, *por*B gene sequence analysis has generally been applied in the study of the evolutionary trajectory, either alone or with other genes of the gonococcus (Golparian, Hellmark, Fredlund, & Unemo, 2010; Unemo, Olcén, Albert, & Fredlund, 2003). Thus, the global efforts to better appreciate the AMR profile and genetic diversities of circulating *N. gonorrhoea* strains remains critical . This will not only be useful for treatment, but would provide useful data for the search of an effective vaccine.

In Ghana and as a microcosm of the African picture, no other work is known to have reported the molecular characteristics of *N. gonorrhoea* other than Attram *et al.,* (2019) and Agbodzi *et al*., (2023). Additionally, there exist no antimicrobial resistance database of gonococcal isolates albeit a few publications (Acheampong *et al*., 2018; Attram *et al*., 2019). However, Ghana's Ministry of Health has continuously updated the syndromic management regimen of gonorrhoea (Ministry of Health Ghana, 2017). Hence, the current study contributes data on the Antimicrobial resistance and molecular characteristics of gonococcal isolates in Ghana to aid in localized syndromic management regimen.

Materials and methods

Secondary gonococcal isolates from the Tamale Teaching Hospital (n=1), Korle-Bu Teaching Hospital, Accra (n=2), and MDS Lancet Laboratories, Accra (n=1) were employed for this work. Gonococcal reference strain ATCC 49226 received from Noguchi Memorial Research Institute, Ghana was included as a positive control.

Media preparation

GC agar supplemented with Vitox (Oxoid, UK) and 5% sheep blood, were prepared following manufacturer's instructions.

Culturing of N. gonorrhoea isolates

Collected *N. gonorrhoea* isolates were inoculated on GC agar supplemented with Vitox and incubated at 37 ^{o}C with moisten cotton in a candle jar as previously described (Duplessis *et al*., 2015).

PCR confirmation and amplification of porB and tbpB genes of N. gonorrhoea

N. gonorrhoea isolates were confirmed by the PCR amplification of two conserved genes, *por*B (737 bp) and *tbp*B (589 bp) as previously described (Martin *et al.*, 2004) with slight modification. Briefly, the crude DNA were obtained by suspending eight to ten colonies in 30 µl Tris-buffer (pH 7.3) and heated at 100 °C for 10 min. A 50 µl final reaction PCR mix of either *por*B or *tbp*B developed in the lab comprised of 5 µl crude DNA, 25 µl of OneTaq 2x Master mix (New England BioLabs Inc.-UK), 6.25 µl each for forward and reverse primers (10 µM) (synthesised by Inqaba Biotec Ltd, SA) of either *por*B or *tbp*B genes, 7.5 µl of nuclease free water was added to make up the 50 µl final volume. Gonococcal reference strain ATCC 49226 was used as a positive control while nuclease-free water was used as negative control in place of DNA. The PCR cycle conditions as previously described (Martin *et al*., 2004) were carried out on peqSTAR 96 Universal Gradient thermal cycler (VWR-USA). Nine microlitre (9 µl) of PCR product mixed with 1 µl 6X gel loading dye (Thermo Fisher Scientific®, USA) were resolved on a 1.4 % agarose gel for 50 min at 80 V. Hundred base DNA ladder (FastRuler™, Thermo Fisher Scientific®, USA) was used as marker. UV trans-illuminator (Cleaver Scientific Ltd, UK) with mounted Canon Power Shot G16 camera (Canon Inc., Japan) was used to visualise the gels.

Antimicrobial resistance (AMR) of confirmed N. gonorrhoea

The resistance profile of the confirmed *N. gonorrhoea* to seven (7) antibiotics (Mast Group Ltd-UK) i.e. penicillin (PG: 10 units), azithromycin (ATH: 15 µg), Ceftriaxone (CRO: 30 µg), Ciprofloxacin (CIP: 5 µg), erythromycin (E: 15 µg), Togamycin (TG: 10 µg) and tetracycline (T: 30 µg) was evaluated using the disk diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2020). For azithromycin, the USA's Centre for Disease Control and prevention (CDC) breakpoint was used to interpret its results.

Sequencing of amplified gonococcal genes

The *por*B and *tbp*B genes PCR amplicons of the confirmed gonococcal isolates and reference strain ATCC 49226 were sequenced by Inqaba Biotec. Ltd, South Africa using Sanger sequencing technology on an ABI Genetic Analyser 3500XL (Thermo Fisher Scientific®, UK). DNA sequence chromatograms were viewed, trimmed, and edited using GENtle (V1.8.0) and Molecular Evolution Genetics Analysis X (MEGA X) software.

NG-MAST analysis

NG-MAST analysis was carried out as described previously (Martin *et al.*, 2004). Briefly, gonococcal DNA sequences were trimmed at consensus regions starting from nucleotide (nt) 445 in reference to *N. gonorrhoea* strain MS11 to yield 490 base pair (bp) for *por*B and for *tbp*B, from nt 1118 reference to strain UU1008 to yield 390 bp. These were submitted to the NG-MAST portal presently held at www.pubmlst.org as ng-mast v2.0 to search against the database's sequence deposits as previously described (Martin *et al.*, 2004). Each sequence (*por*B or *tbp*B) of the same isolate describes a locus type, while a combination of the loci types describes a Sequence Type (ST).

*N. gonorrhoea por*B *sequence analysis*

The *por*B gene sequence lengths were trimmed to yield 510 bp based on readability of the chromatograms (Liao *et al.*, 2009) starting from nucleotide 1990471 to 1990980 relative to *Neisseria gonorrhoea* strain WHO N genome assembly, chromosome 1 (Accession No.: LT591910.1). The National Centre for Biotechnology Information's (NCBI) online Basic Local Alignment Search Tool (BLAST) was used to compare sequence similarities with other *N.*

gonorrhoea sequence deposits and to identify their isoforms. Subsequently, intra sequence variation was determined using pairwise alignment on GENTle (V1.8.0). A global haplotype analysis network was constructed on Population Analysis with the Reticulate Tree (PopArt v.1.7) software using the Theoretical Computer Science (TCS) algorithm to visualise the genealogical relatedness of the gonococcal isolates with 22 others found worldwide. MEGA X software was employed to align sequences using ClustalW and to draw the phylogenetic tree using the maximum composite likelihood model while employing 1000 bootstrap replications to test the confidence of the tree.

Results

PCR confirmation of presumptive N. gonorrhoea isolates

All four (4) secondary isolates were confirmed as *N. gonorrhoea* using *por*B and *tbp*B genes. The PCR-confirmed gonococcal isolate from Tamale was assigned S2, whiles the other three confirmed isolates from Accra were assigned S1, S3, and S4 (See **Figure 1**).

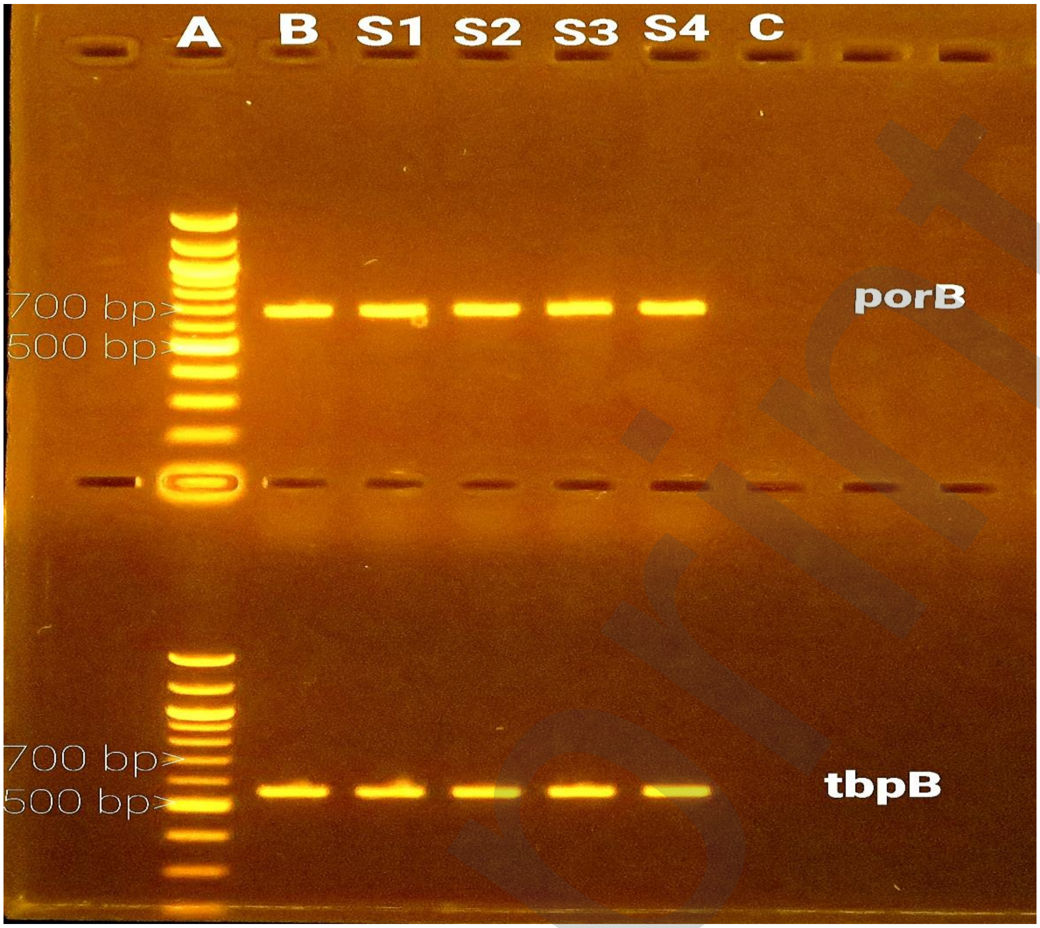

Figure 1. PCR confirmation of gonococcal isolates S1, S2, S3, and S4 showing the amplification of *por*B (737 bp) top and *tbp*B (589 bp) bottom. A-100 bp DNA markers

B-*N. gonorrhoea* reference strain (ATCC 49226) as positive control. C- Negative control.

Antimicrobial resistance of confirmed N. gonorrhoea isolates

All four (4) gonococcal isolates exhibited resistance or intermediate susceptibility to all the seven (7) antibiotics tested except ceftriaxone. Ceftriaxone resistance was observed in isolate S4. All four isolates revealed a multiple antibiotic resistance index of > 0.5. **Table 1** shows the antimicrobial resistance (AMR) profile of the gonococcal isolates against the seven tested antibiotics. **Figure 2** illustrates the percentage resistance to the seven antimicrobials by the four gonococcal isolates.

Table 1. Antimicrobial resistance profile of four *N. gonorrhoea* isolates against CLSI and CDC's breakpoints interpretation of seven antibiotics. R, S and I represent resistance, susceptible and intermediate susceptibility respectively. Whereas T- Tetracycline, PG-Penicillin, E- Erythromycin, CRO- Ceftriaxone, CIP-Ciprofloxacin, ATH-Azithromycin, TG-Togamycin. MAR denote multiple antibiotic resistance index; a ratio of resistance antibiotics to the total number of antibiotics evaluated.

Antibiotics (7)	**S1**	**S2**	**S3**	**S4**
T (30 µg)	R	R	R	R
PG (10 U)	R	R	R	R
E (15 µg)	R	R	R	R
CRO (30 µg)	S	S	S	R
CIP (5 µg)	R	I	I	R
ATH (15 µg)	I	I	I	R
TG (10 µg)	R	R	R	R
MAR Index	**0.71**	**0.57**	**0.57**	**1.00**

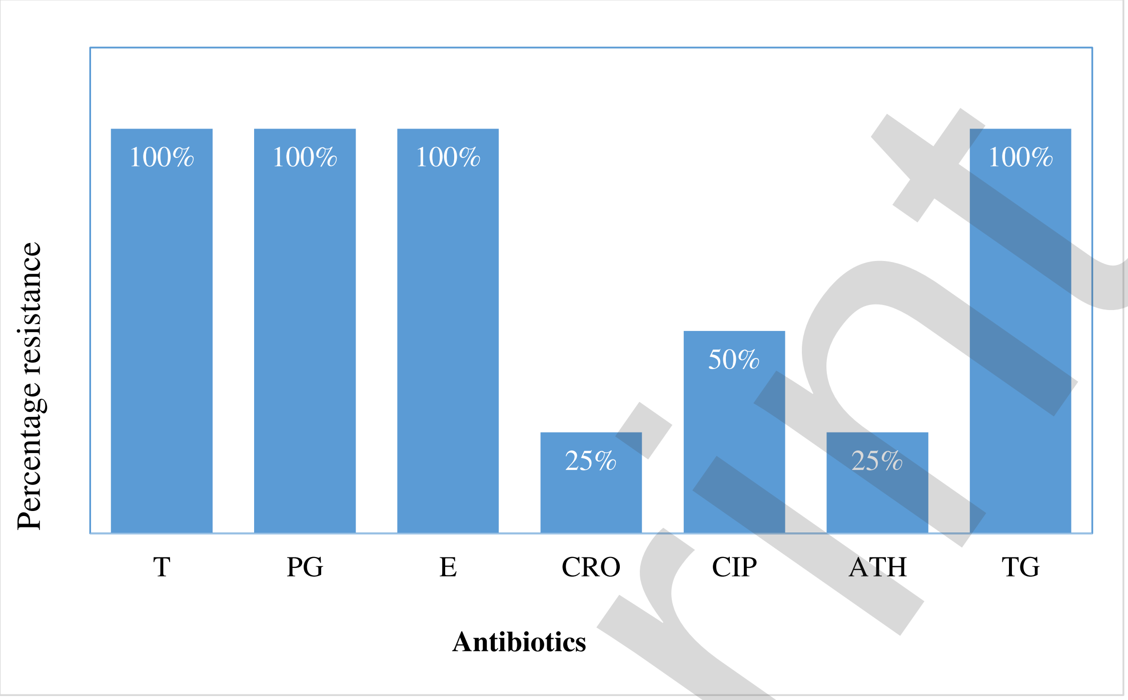

Figure 2. Percentage resistance to seven antibiotics by four *N. gonorrhoea* isolates. (Where T-Tetracycline, PG-Penicillin, E-Erythromycin, CRO-Ceftriaxone, CIP-Ciprofloxacin, ATH-Azithromycin and TG-Togamycin).

Molecular characterisation of N. gonorrhoea using porB and tbpB genes

Following two DNA sequencing attempts of the four gonococcal isolates and the reference strain, only the sequence chromatograms of S2 and S3 were readable for both genes (i.e. *por*B and *tbp*B). Therefore, sequences of S2 and S3 were trimmed appropriately for downstream molecular characterisation.

Molecular typing (NG-MAST) of N. gonorrhoea

Isolate S3 was identified as NG-MAST Sequence Type 211 (ST211). A locus search of S2 *tbp*B gene identified the allele as *tbp*B1844. However, its *por*B locus search revealed a 40% similarity with alleles in the database, hence, its Sequence Type could not be determined.

Molecular characteristics of N. gonorrhoea using porB (510 bp) gene

BLAST search on NCBI's GenBank with S3 (*por*B) revealed 100% identity with 25 deposited gonococcal sequences. These includes WHO genome assemblies' N (Accession: **LT591910.1**) and G (Accession: **LT591898.1**). Other 100% identities were partial coding sequences of the gene such as **KF421819.1** (Germany), **GQ289460.1** (China), **EU530748.1** (Russia), **AF090820.1** (Kenya) and **AF304403.1** (USA). On the other hand, S2 (*por*B) recorded a highest identity of 99.61% with three deposited sequences with the following accession numbers; **AF200756.1** (USA), **AF090810.1** (Kenya), and **AF090809.1** (Kenya). Both S2 and S3 sequences were identified as *por*B 1A allele and were assigned GenBank accession numbers **MZ313863** and **MZ313864** respectively. Fifteen singleton variable sites were identified between S2 (**MZ313863)** and S3 (**MZ313864)** as confirmed by the pairwise alignment shown in **Figure 3.**

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S2(MZ313863)  TGAAAGCCAT TTGGCAGTTG GAACAAAAAG CCTACGTCAG CGGTACTGAC ACAGGCTGGG GCAACCGCCA ATCCTTCATC
S3(MZ313864)  ·········· ·········· ·········· ·········· ·······A·A GA········ ···G······ ··········
Identity      ********** ********** ********** ********** ******* *    ******** *** ****** **********

S2(MZ313863)  GGTTTGAAAG GCGGATTCGG TAAAGTGCGC GTCGACCGTT TGAACAGCGT CCTGAAAGAC ACCGGCGGCT TCAATCCTTG
S3(MZ313864)  ·········· ····C····· ·········· ····G··A·· ······A·A· ·········· ····A····· ··········
Identity      ********** **** ***** ********** **** ** ** ****** * * ********** **** ***** **********

S2(MZ313863)  GGAGGGTAAA AGCTACTATT TGGGTTTAAG CAACATTGCC CAACCCGAAG AACGCCACGT TTCCGTACGC TACGATTCTC
S3(MZ313864)  ·········· ·········· ·········· ·········· ·········· ·········· ·········· ··········
Identity      ********** ********** ********** ********** ********** ********** ********** **********

S2(MZ313863)  CCGAATTTGC CGGCTTCAGC GGCAGCGTGC AATACGTGCC TAACGACAAT TCGGGCAAAA ATCACAGCGA ATCTTACCAT
S3(MZ313864)  ·········· ·········· ········A· ·········· ·········· ·········· ···G······ ··········
Identity      ********** ********** ******** * ********** ********** ********** *** ****** **********

S2(MZ313863)  GCAGGCTTCA ACTACAAAAA CAGCGGCTTC TTCGTGCAAT ATGCCGGCTT CTATAAAAGA CATAATTACA CGACTGAGAA
S3(MZ313864)  ·········· ·········· ·········· ·········· ·········C ·········· ·········· ··········
Identity      ********** ********** ********** ********** *********  ********** ********** **********

S2(MZ313863)  ACACCAGGTT CACCGTTTGG TCGGCGGTTA CGACCATGAT GCCCTGTACG CTTCCGTAGC CGTACAGCAA CAAGACGCGA
S3(MZ313864)  ·········· ·········· ·········· ·········· ·········· ·········· ·········· ··········
Identity      ********** ********** ********** ********** ********** ********** ********** **********

S2(MZ313863)  AATTGACTTG GCGCAACGAT AATTCGCACA
S3(MZ313864)  ·········· ·A········ ··········
Identity      ********** * ******** **********
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Figure 3. Pairwise sequence alignment of S2 (MZ313863) and S3 (MZ313864) (510 bp) shows fifteen singleton variations. (* indicates similarities).

Molecular characteristics (Haplotype network analysis)

The global haplotype analysis conducted shows the relationship between S2 (**MZ313863)** and S3 (**MZ313864)** as well as with other isolates found worldwide. S3 (**MZ313864)** clustered with previously reported haplotypes from China, UK, Russia, USA and Kenya whilst S2 (**MZ313863)** is distantly related to the cluster as seen in **Figure 4.**

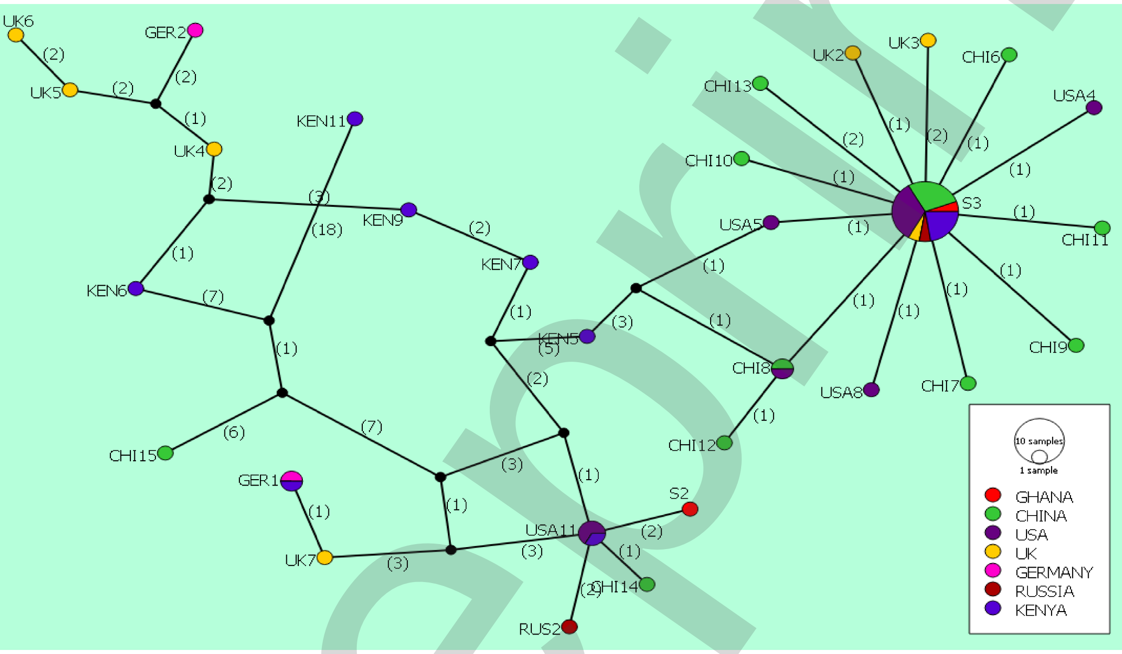

Figure 4. Global haplotype network of *N. gonorrhoea por*B gene (510 bp) showing S3 (MZ313864) clustering with previously reported haplotypes and S2 (MZ313863) distantly related to this cluster. Numbers in brackets indicated on the lines represent the number of mutations between haplotypes. Coloured circles represent haplotypes previously identified in other countries shown in the key. Smaller black circles/dots represent missing haplotypes.

Phylogenetic analysis of N. gonorrhoea using porB (510 bp) genes

Phylogenetic analysis of the two haplotypes identified in this study with 22 other sequence deposits from GenBank based on *por*B gene (510 bp) showed S2 (**MZ313863)** forming a clade with GenBank sequences from the USA (**AF200756.1**) and Kenya (**AF090810.1**). The *por*B sequence of S3 (**MZ313864)** formed a monophyletic clade with sequence deposits from Germany (**KF421819.1**), China (**EU719208.1**, **HM451254.1**), Russia (**EU530748.1**), Kenya (**AF090816.1**) and USA (**AF200747.1**) as shown in **Figure 5.**

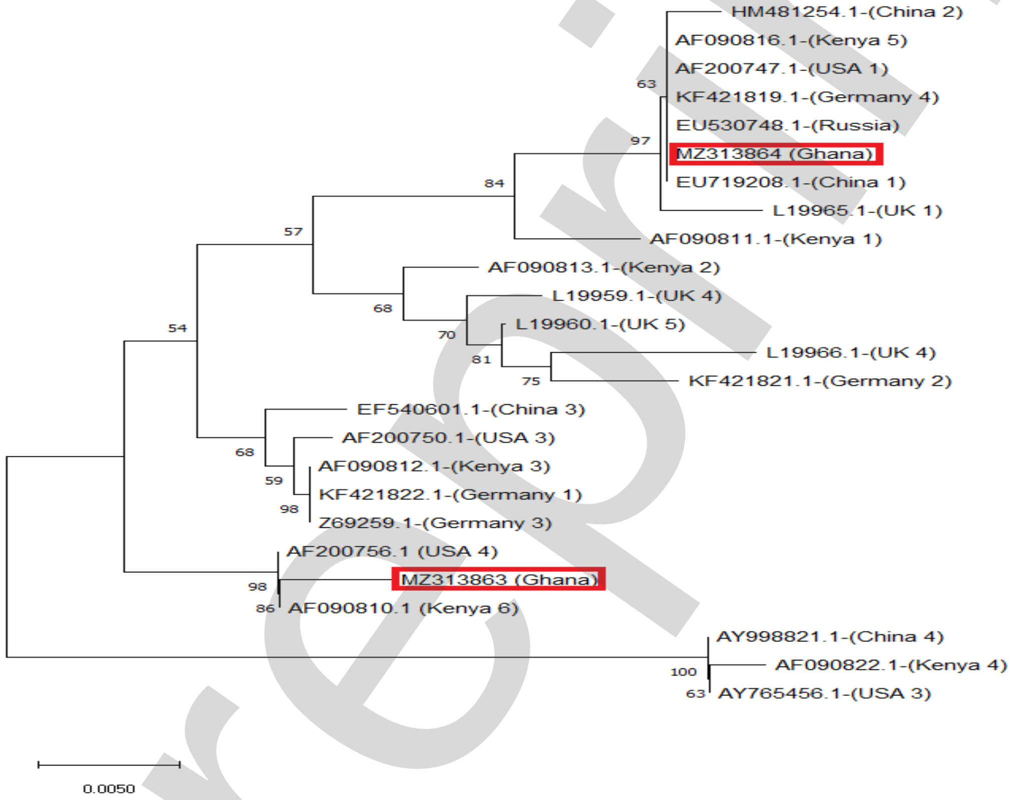

Figure 5. Genetic phylogram showing evolutionary relatedness of isolates of *N. gonorrhoea* (*por*B gene, 510 bp) from this study relative to others found elsewhere. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.

Discussions

Antimicrobial resistance profile of the N. gonorrhoea isolates

The employed antibiotics for antimicrobial testing are presently in use or had previously been used in Ghana and elsewhere to treat gonorrhoea. The four antibiotics (Tetracycline, Penicillin, Erythromycin and Tagomycin) resisted by all the isolates in this study, indicating 100% multi-drug resistance, which was previously reported in Ghana (Acheampong *et al*., 2018; Duplessis *et al*., 2015) and elsewhere (Azizmohammadi & Azizmohammadi, 2016; Hailemariam, Abebe, Mihret, & Lambiyo, 2013). This indicates that these antibiotics are still resisted by circulating *N. gonorrhoea* after years of its withdrawal as therapeutic agents. The recorded Resistance (50%) and intermediate susceptibility (50%) to ciprofloxacin has also been recorded previously (Acheampong *et al.,* 2018; Duplessis *et al.,* 2015) which supports its withdrawal as first-line treatment regimen by the Ghana Health Service in 2018. Nonetheless, ciprofloxacin is still in use in some parts of Ghana to treat gonorrhoea. This ability of *N. gonorrhoea* to resist fluoroquinolones (ciprofloxacin) is rendered by *gyrA* mutations which reduces its binding affinity (Belland, Morrison, Ison, & Huang, 1994; Unemo & Shafer, 2014). Resistance and reduced susceptibility to azithromycin by *N. gonorrhoea* as observed in this study was first reported in Argentina in 2001 (Galarza *et al.,* 2009) and became a global concern by 2010 (Berçot *et al*., 2014; Chisholm, Dave, & Ison, 2010; Olsen *et al.*, 2013). Resistance to azithromycin has previously also been reported in Ghana (Attram *et al.,* 2019). However, it is still recommended in the current globally recommended dual-treatment regimen of gonorrhoea with ceftriaxone (Kidd & Workowski, 2015). Transmutations in either the *mtrR, erm* or *mef* genes which encodes the efflux pump that results in the overexpression of the efflux pump systems in *N. gonorrhoea* has been linked with reduced sensitivity to azithromycin (Allen *et al.,* 2011, 2014; Golparian, Shafer, Ohnishi, & Unemo, 2014).

Treatment efficacy of the extended-spectrum cephalosporins (ESCs) including ceftriaxone has been widely reported hence its current recommendation for the treatment of gonorrhoea (Kirkcaldy *et al.,* 2016; Lefebvre *et al.,* 2018; Nakayama *et al.,* 2016; Ohnishi, *et al.,* 2011; Unemo & Nicholas, 2012). However, treatment failures and reduced susceptibility recorded in this study had earlier been reported (Ohnishi *et al.*, 2011; Unemo *et al.*, 2012) and recently in Ghana (Acheampong *et al.,* 2018). The primary resistance factor to ESCs has been linked to the alleles of mutant mosaic penicillin-binding protein two (PBP2) and a non-mosaic PBP IX harbouring P551L substitution (Gianecini, Oviedo, Stafforini, & Galarza, 2016). Reported resistance to the last resort ESC antibiotics is a threat to treatable gonorrhoea. The Tamale isolate S2 (**MZ313863)** showed same antibiotic resistant profile of the tested antibiotics to the Accra isolate S3 (**MZ313864).** The other Accra isolates (S1) and (S4) showed different resistant profile, with S4 recording resistance to all the tested antibiotics. The difference in antibiotic resistant profiles recorded by all three Accra isolates showcase the heterogeneity of the circulating strains in Ghana. The outcome of the antibiotic profile of this study buttresses the widespread apprehension about *N. gonorrhoea'*s ability to develop into a “superbug” despite the small number of isolates studied. This is particularly important for developing countries where syndromic management is the official recommended means of treating gonorrhoea and other STIs.

Molecular characteristics of N. gonorrhoea isolates

The NG-MAST sequence typing revealed two genotypes; type 211 (ST211) identified with isolate S3 (**MZ313864**), which has previously been reported in the UK, Germany and New

Zealand has been associated with decrease or resistance to ciprofloxacin (Golparian *et al.,* 2014; Palmer, Young, Martin, Ison, & Spratt, 2005). Locus 1844 (*tbp*B gene) identified with S2 (**MZ313863**) was found with only one NG-MAST sequence type, i.e. ST10251, indicating that it may not be a widespread allele. Even though its *por*B sequence returned a 40% similarity with other sequences in the NG-MAST database, it was found to be 99.61% similar to other sequence deposits in NCBI's GenBank. This indicates that not all sequence deposits in the NG-MAST database have been deposited in GenBank and vice versa. This situation calls for a holistic consolidation of gonococcal data globally. Neither the identified NG-MAST ST211 nor the *tbp*B allele type 1844 in this study had previously been reported in Ghana. S2 (**MZ313863)** and S3 (**MZ313864**) identified as *por*B IA are associated with disseminated gonorrhoea (Chen & Seifert, 2013) which may cause meningitis, endocarditis, arthritis, septicaemia among other deleterious complications (Unemo *et al*., 2019).

The significant genetic variation between S2 (**MZ313863**) and S3 (**MZ313864**) despite similarity in the antibiotics resistant profile recorded in this study, points to a wide genetic diversity of gonococcal strains in Ghana. The origin of the observed intercontinental genealogical relatedness of haplotype S3 (**MZ313864**) is not readily known. While the *por*B sequence of S2 (**MZ313863**) showed (99.61% similar to three others) perhaps a novel strain, more gonococcal samples need to be studied in the Tamale Metropolis where it was isolated to confirm and ascertain its spread. This study corroborate earlier study in southern Ghana (Attram *et al.,* 2019) where varied strains of *N. gonorrhoea* were identified. However, the relation of the present isolates to Attram *et al.,* (2019) could not be ascertain as the latter had not deposited the encountered sequences on any genetic database at the time of this study.

The fifteen singleton variable sites observed between the two isolates is evident in their distanced clustering in the global haplotype analysis and their formation of monophyletic groups. Relative to the other isolates found globally, it suggests either these strains were transmitted through travels or developed independently as a result of undergoing similar stress. The evolutional history also indicated that S2 (**MZ313863**) shared a common ancestor before at least six-ancestry evolution that resulted in the clade involving S3 (**MZ313864**). To emphasise this, continuous assessment of local circulating strains of *N. gonorrhoea* in Ghana, especially in the northern part of the country would enhance the knowledge of circulating gonococci strains. This information would help inform appropriate local therapeutic regimens and the global effort in vaccine development.

Conclusion

This study recorded two strains of *N. gonorrhoea* from the four molecularly confirmed *N. gonorrhoea* isolates from Accra and Tamale. Multidrug resistance was observed, with all four *N. gonorrhoea* isolates showing resistance against at least four antibiotics. One of the Accra isolates was resistant to all seven antibiotics. There’s therefore an urgent need for continues surveillance and an expedite action to develop gonorrhoea vaccine before widespread deaths due to untreatable gonorrhoea emerges.

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Conflict of interest: None

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Authors Contributions: HS Musah; Methodology, Data collection, Data analysis, and Writing-Original draft, F Addy; Supervision, validation, writing – review & editing of manuscript and OA Dufailu; Conceptualization, Supervision, Methodology, Validation, Writing – review & editing of manuscript.

Ethical approval: The University of Health and Allied Science's ethical clearance committee approved the research with protocol identification number **UHAS-REC A.12 (3) 18-19.**

Data Availability statement: Molecular data has been deposited on Genbank with accompanying accession numbers as stated earlier.

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