Identification of multidrug-resistant salmonella plasmid variants using polymerase chain reaction (PCR) based replicon typing technique in Ghana

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Abstract

Introduction: *Salmonella* infections cause significant morbidity and mortality, especially in resource-limited countries. The situation is worsened by widespread presence of multidrug resistant (MDR) strains, largely encoded on conjugative plasmids, but with little knowledge about how these plasmids are identified especially in low-middle income countries. We present findings of various plasmid variants possibly encoding MDR *salmonella* from Ghana.

Methods: this was a cross-sectional study involving individuals suspected of having *salmonella* infection presenting at two major hospitals in Ghana. Blood, stool and oropharyngeal specimens (OPS) were taken from consenting individuals between May, 2016 and January, 2018. Identification of *salmonella* was done using standard microbiological procedures. Total DNA was extracted from MDR *salmonella* isolates and PCR-based replicon typing (PBRT) performed using 30 replicons representative of the major incompatibility groups among Enterobacteriaceae.

Results: of 2,376 samples collected, 101 (4.3%) *salmonella* were isolated. Multidrug-resistant *salmonella* was detected in 34 (33.7%) strains; S. Typhi (67.6%), NTS (32.4%). Four different incompatibility (*Inc*) groups were identified by PBRT. Eleven *S. Typhi* bacteremic isolates (32.4%), harboured plasmids with *Inc* group HI1 of target size 534bp. The most predominant replicon (13/34; 38.2%) belonged to *Inc*U plasmid. Non-typhoidal *salmonella* harboured 1 rare *Inc*X2 plasmid and 8 *Inc*FIIS plasmids known to encode resistance to carbapenems, colistins and several virulence genes.

Conclusion: this study shows presence of circulating plasmid variants likely to confer MDR in *salmonella* from clinical isolates. This is the first time Ghana has reported 3 (*Inc*U, *Inc*FIIS, *Inc*X) of 4 variants and these are similar to those circulating in Africa but one (*Inc*FIIS).

Introduction

Salmonella infection has been a global problem for centuries. It is among the top four important bacterial agents of diarrhoeal illness reported globally [**1**], and caused by one of several serovars of gram negative facultative anaerobic *Salmonella* bacteriae. Cases of the infection are usually mild, especially in the case of NTS infection. However, it can be life-threatening, depending on host immune factors present and the type of serovar involved [**2**]. Generally, the disease is frequently characterized by abdominal pain, fever, diarrhoea, nausea and/or vomiting [**3**]. In Ghana, the most predominant *Salmonella* infections are caused by *Salmonella enterica* serovar Typhi (S. Typhi), and two other non-typhoidal *Salmonella* (S. Typhimurium and S. Enteritidis) [**4**, **5**]. The emergence and spread of resistant strains of the organism have posed several health and economic threats globally. Of note is the occurrence of multidrug-resistant (MDR) *Salmonella* strains, defined as resistance to at least three classes of anti-*Salmonella* drugs [**6**, **7**]. These MDR strains are known to be encoded on conjugative plasmids [**8**, **9**], however, knowledge about the different variants of plasmids associated are poorly understood in Ghana. In the meantime, plasmids increase bacterial genetic diversity through the acquisition and loss of genes, especially those responsible for antibiotic resistance and/or virulence within the bacterial population [**10**]. A special property exhibited by plasmids which is more suitable for their classification is Incompatibility (*Inc*) grouping [**11**]. Incompatibility is a manifestation of relatedness, sharing common replication controls/equipartitioning elements. Thus, plasmids that are incompatible with each other are assigned to the same incompatibility group. The role of antimicrobial resistant plasmids is extremely vital as it allows for diffusion of these MDR *Salmonella* strains into different environments to be traced [**11**]. Knowledge of specific plasmids associated with antimicrobial resistance and virulence in bacterial host are able to be monitored when identified early [**12**]. While low-middle income countries of Africa (including Ghana) and Asia bear a greater brunt of the disease burden, very little is known about the various plasmid variants existing in

those areas and their consequential diffusion within the environment. This study therefore sought to identify plasmids likely to be encoding MDR *Salmonella* into incompatibility groups (*Inc* groups) using a multiplex polymerase chain reaction (PCR) based replicon-typing approach for the first time in Ghana.

Methods

Study design and area: this was a cross-sectional study conducted from May 2016 to January 2018 as part of a larger study that is examining the burden of severe typhoid in sub-Saharan Africa involving six countries (Ghana, Burkina Faso, Democratic Republic of Congo, Ethiopia, Nigeria and Madagascar). However, the present report is only from Ghana. In Ghana, there were two participating major hospitals, one rural (Agogo Presbyterian Hospital (APH)), and the other a major referral hospital referred to as Komfo Anokye Teaching Hospital (KATH) in Kumasi. Census data and hospital records were used to define the study catchment areas. The Agogo Presbyterian hospital typically serves patients living within and around the Asante-Akim North District (AND) of Ashanti region whereas KATH provides services for patients living within Kumasi metropolis (KM) and beyond.

Study population and sample size estimation: the study population comprised patients of all ages and gender attending the selected local health facilities presenting with symptoms of *Salmonella* infection such as fever (≥ 38°C tympanic and/or ≥ 37.5°C axillary) of at least three days' duration, headache, stomach cramp and nausea. The minimum sample size expected was 150 based on 6.5%, 2.3% and 1.3% prevalence of *Salmonella* in blood, stool and oropharyngeal specimens (OPS), respectively using precision of 5% and standard normal deviation of 1.96 corresponding to 95% confidence interval [**13-15**]. However, we oversampled from the target population to take care of any population variation that may have inadvertently been ignored and to allow for adequate study power. In all a total of 2,376 samples was used for the study.

Ethical clearance: the consent process was at different levels. First we sought permission from all patients and their relatives before samples were taken from them. Children older than 7yrs but younger than 18yrs gave their assent together with parental/guardian consent before they were enrolled into the study. The study was explained to participants and their consent sought by means of signature and/or thumb printing. The main study protocol was reviewed and approved by the Committee for Human Research Publications and Ethics (CHRPE) at the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST).

Sample collection and transportation: eight milliliters (adult) and 3ml (pediatric) of venous blood were collected using a 23G and 21G butterfly needles, respectively and inoculated directly into a blood culture bottle (Becton Dickinson (BD), Franklin Lakes, NJ USA). Stool samples were collected into 30ml sterile screw cap stool containers. About 1g (loopful) of it was inoculated into selenite broth on site before transportation. Again, OPS were collected from recruits using sterile floq swabs. The stool specimen and OPS were transported immediately from both sites to Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR) laboratory at 4°C whiles the blood culture bottles were transported at room temperature.

Bacteria identification from clinical specimens

Bacteria cultures: a total of 2,376 samples (blood-1400, stool-418 and OPS-558) were collected from study participants from both hospitals. Variance in sample numbers in the three samples was as a results of unwillingness of study participants to provide stool and OPS specimens more readily compared to the blood samples. Blood samples collected into culture bottles were incubated in BACTEC™ 9050 blood culture system at 35°C for five days or until positive signal was detected. Direct stool and OPS were partly plated on xylose lysine deoxycholate (XLD) agar and incubated overnight (18-24hours) at 35-37°C. Other portions of stool samples (already inoculated in selenite broth) and OPS enriched in selenite broth were also incubated overnight at 35-37°C for recovery of injured *Salmonella*. Inoculated stool specimen and OPS were streaked onto XLD agar after 24hours of incubation using sterile 10µl bacteriological loop.

Identification of *Salmonella*: positive blood culture samples were plated onto 3 standard growth media: blood agar (BA - Columbia agar base supplemented with 5% sheep blood), chocolate agar (CA) and macConkey (Mac) agar (BD, USA) under sterile working condition. MacConkey and blood agar were incubated overnight under aerobic conditions at 35-37°C whiles CA plates were incubated in candle jar for microaerophilic condition at the same temperature. Any bacterial growth on all 3 media observed as small creamy non-hemolytic colonies on BA and CA, and its corresponding Mac plate giving small transparent non-lactose fermenting colonies were noted for further testing. On XLD agar, any pink colonies with black pigments from stool and OPS were investigated for *Salmonella* identification. Pure colonies were sub-cultured from XLD onto blood agar for biochemical tests. Gram negative short rods gave a presumptive identification. Biochemical tests were performed as follows: triple sugar iron agar (TSI) – red slope on yellow butt (R/Y), with strong/weak hydrogen sulphide (H_2S) production and gas/no gas seen; negative indole and urease tests; positive or negative citrate test and SIM test - sulphide (+/-), indole (-) and motility (+). Again, latex agglutination test (Oxoid, UK) was performed on the presumptive *Salmonella* isolates to check for agglutination after emulsifying pure colonies in commercially prepared latex antisera for 10 seconds. Confirmation of *Salmonella* was done by Analytical Profile Index (API) 20E (Biomerieux, France). Finally, serotyping was performed to identify which serovar of *Salmonella* was invloved.

Antimicrobial susceptibility testing: the antibiotics chosen for testing were based on current treatment regimens for *Salmonella* infections as well as clinical and laboratory standards institute [**16**] standards. Susceptibility to ampicillin (10µg), amoxiclav (amoxicillin & clavulanic acid; 20/10µg), ceftriaxone (30µg), trimethoprim/sulfamethoxazole (1.25/23.75µg), ciprofloxacin (5µg), gentamicin (10µg), tetracycline (30µg), chloramphenicol (30µg), ceftazidime (30µg), cefotaxime (30µg) and nalidixic acid (30µg) was tested on Mueller-Hinton agar (BD, USA) using the Kirby-Bauer disc diffusion method. Isolates resistant to 3 or more classes of antibiotics were regarded as MDR.

Quality control: *escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028 were set up together with the test organisms to control media, biochemical tests and potency of antibiotic discs.

DNA extraction: total DNA was extracted on all MDR *Salmonella* isolates from blood, stool and OPS using spherolyse DNA extraction kit according to manufacturer's instructions (Hain-life Science, Germany).

Plasmid incompatibility profiling: PCR-based replicon typing (PBRT) method was used to detect plasmid variants encoding MDR in *Salmonella* isolates using PBRT 2.0 kit (Diatheva, Italy). This kit contains novel set of 8 panels (designated as M1, M2, M3, M4, M5, M6, M7 and M8) of specific PCR assays optimized to perform 8 multiplex PCRs for the amplification of 30 replicons (**Table 1**). These replicons/plasmid variants represent the major plasmid incompatibility groups and replicase genes identified on resistance plasmids circulating in Enterobacteriaceae [**17**,**18**]. Total DNA preparations were used as templates along with incompatibility groups within 8 panels in order to amplify specific target regions (**Table 1**).

PCR mastermix preparation: for 1 sample, 23.8µl PCR mix from tube M1 was pipetted into an empty sterile 1.5ml Eppendorf tube. 0.2µl DNA polymerase was added and mixed gently by vortexing. Two extra 1.5ml tubes each containing 23.8µl and 0.2µl PCR mix from tube M1 and DNA polymerase, respectively were added to serve as positive and negative control tubes. The same procedure was performed for the rest of the PCR mixes (M2 - M8). One microlitre of template DNA and controls were added to their respective PCR mix tubes containing DNA polymerase.

Polymerase Chain Reaction (PCR): amplification of targeted replicons within the extracted samples was performed by conventional multiplex PCR using a 96-well verity thermal cycler (Applied Biosystems, USA). The following amplification conditions were used to run all the 8 panels (M1 - M8): 1 cycle of initial denaturation at 95°C for 10 minutes, 30 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 30 seconds, elongation at 72°C for 1 minute and final extension cycle at 72°C for 5 minutes. The final amplicons were kept on hold at 4ºC prior to collection for agarose gel electrophoresis.

Agarose gel electrophoresis: ten microlitres each of the PCR amplicon was mixed with 2µl loading buffer (bromophenol blue) and the final volume loaded onto a 2.5% agarose gel containing 2µl of ethidium bromide. Positive and negative controls were also loaded for each set (M1-M8), including 12µl of molecular ladder specific for low range (100-1000bp). Electrophoresis was initiated for 2 hours

at 100V (**Figure 1**). Visualisation of the gel was done by ultraviolet (UV) transilluminator and captured onto a desktop computer connected to it using Infinity® software.

Statistical Analysis: a paper-based questionnaire was administered to record each participant's biodata. Blood, stool and OPS were taken from each consented participant and those positive for MDR *Salmonella* were subjected to PBRT to determine the presence of plasmid variant(s) encoding MDR. Data were collected, entered into Microsoft excel and exported to STATA version 12 (Stata Corp, USA) for analysis. Descriptive statistics was used to summarize the distribution of various variables into tables and graphs. Differences between discrete variables were analysed using chi-square with 95% confidence interval and 5% margin of error.

Results

General characteristics of the study population: during the study period of May, 2016 to January, 2018, a total number of 4,561 participants were approached and screened for eligibility. However, 1,400 were eligible and consented to take part in the study (**Table 2**). Overall, a total number of 2,376 samples were collected from the two study sites. One thousand four hundred blood, 418 stool and 558 OPS representing 58.9%, 17.6% and 23.5%, respectively were obtained from the study participants. At KATH alone, of 680 samples received, 364 (53.5%) were blood samples for culture, 159 (23.4%) were stool and 157 (23.1%) OPS specimens. Of 1,696 samples collected from APH, blood culture samples were 1,036 (61.1%), 259 stool (15.3%) and 401 OPS (23.6%).

***Salmonella* identification from clinical specimens:** of 2,376 samples collected and analysed, 101 (4.3%) confirmed *Salmonella* isolates were obtained from blood (n=84), stool (n=13) and OPS (n=4). Salmonellae were classified either as serovar Typhi or Non-typhoidal *Salmonella* (NTS). Of 101 *Salmonella* isolates, 68 (67.3%) S. Typhi and 16 (15.8%) NTS were isolated from blood; 3 (2.9%) S. Typhi and 10 (9.9%) NTS from stool; and 1 (0.9%) S. Typhi and 3 (2.9%) NTS isolated from OPS. Children (between <1 to 19 years) were predominantly infected with both S. Typhi (68/72) and NTS (27/29) than adults.

Antimicrobial susceptibility profile of *Salmonella* isolates: all *Salmonella* isolates from blood, stool and OPS were tested to determine their antimicrobial susceptibility to selected antibiotics (**Table 3**). Trimethoprim/Sulfamethoxazole showed highest rate of resistance (27/72; 37.5%) in S. Typhi whiles no resistance was observed in gentamicin and 2 cephalosporins (ceftazidime and ceftriaxone). Conversely, among those NTS infected samples, ampicillin recorded the highest resistance (14/29; 48.3%) whiles ciprofloxacin, gentamicin and 3rd generation cephalosporins showed excellent activity (**Table 3**). Thirty-four (33.7%) of the 101 *Salmonella* isolates exhibited multidrug-resistance. Overall mean age (mean ± SE) of individuals infected with any MDR *Salmonella* was 6.5 ± 1.0 (p = 0.0001). Mean ages of individuals infected with MDR S. Typhi and NTS were 7.9 ± 1.2 and 2.5 ± 1.7 (p = 0.0001), respectively. *S. Typhi* MDR (23/34; 67.6%) was twice as many as non-typhoidal *Salmonella* MDR (11/34; 32.4%). The multidrug-resistance distribution of the *Salmonella* identified showed 16 (47.1%), 11 (32.3%) and 7 (20.6%) isolates were resistant to 3, 4 and 5 antibiotics, respectively (**Table 4**).

Incompatibility profiling: PCR-based replicon typing produced 4 different plasmid incompatibility (*Inc*) groups that could be encoding *Salmonella* MDR in the clinical samples tested. Two of these *Inc* groups (*Inc*HI1 and *Inc*U) were detected in MDR S. Typhi from the blood. Specifically, 11 (32.4%) isolates, harboured *Inc*HI1 of target size 534bp (**Figure 1** A). Majority (13/34; 38.2%) of the MDR S. Typhi isolates tested possessed replicon of size 843bp belonging to *Inc*U (**Figure 1** D). Overall mean ages of individuals with MDR S. Typhi possessing *Inc*HI1 and *Inc* U were 8.8 ± 1.8 and 9.5 ± 1.6, respectively. Individuals between the ages of 6-10 were mostly infected with both *Inc*HI1 and *Inc* U S. Typhi (**Table 5**). Male children (8/11; 72.7%) infected with S. Typhi were predominantly found to be encoding plasmids with *Inc* HI1 replicons than female children (3/11; 27.3%) (**Table 5**). Patients who visited APH were significantly infected with MDR *Salmonella* from the *Inc* HI1 plasmid group (p=0.01) (**Table 5**). Unlike S. Typhi, NTS bacteremia yielded 8 (23.5%) isolates belonging to *Inc* FIIS of target size 260bp (**Figure 1** B) with female children of age group <1 – 5 years mostly affected (5/8; 62.5%) (**Table 5**). Again, children between the ages of <1 – 5 years were found to be significantly prone to harbour *Inc*FIIS plasmid than the older groups (p=0.01)

(**Table 5**). Incompatibility group *Inc* X2 was detected in only 1 female NTS isolate (**Figure 1** C). Multireplicon isolates were detected in 4 of the samples tested: 1 isolate (NTS) harboured both *Inc*X2 and *Inc*FIIS plasmids whereas 3 (all S. Typhi) harboured plasmids in the *Inc*U and *Inc*HI1 groups (**Table 6**).

Discussion

This study found appreciable numbers of MDR cases of both *Salmonella* Typhi and non-typhoidal *Salmonella* in the two study populations. Four plasmid variants were identified in MDR *S. Typhi* (*Inc*HI1 and *Inc*U) and NTS (*Inc*FIIS and *Inc*X2). Children less than 15 years were significantly associated with MDR *Salmonella* belonging to *Inc*U and *Inc*FIIS plasmids. Again, individuals seeking medical care in rural Ghana were found to be more likely to possess *Inc*HI1 plasmid than those from the urban area. Globally, *Salmonella* infection poses a significant public health threat. Enteric fever disease occurs throughout the tropics and sub-tropics of Africa, Asia and South America, with a high disease burden in South-East Asia. However, NTS has a high burden in sub-Sahara Africa with moderate infection in South-East Asia [**19-21**]. In Ghana, annual cumulative *S. Typhi* incidence for children <5 years was reported in a study as 3.3 per 1000 and that of NTS as 25 per 1000 population, giving a clear indication of an alarmingly high rate of *Salmonella* infection in the country [**22**].Antibiotic resistances in all genera of disease-causing bacteria have increased tremendously in recent times [**7**, **23**]. There is therefore the need by the scientific community to undertake routine surveillance of microbial populations to determine the extent of these resistances. Inexpensive first line drugs (ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole) used for *Salmonella* treatment in the past are no longer effective, paving way for third generation cephalosporins and fluoroquinolones [**24**, **25**]. In this study, 101 clinical isolates of S. Typhi (71.3%) and NTS (28.7%) were investigated for their susceptibility to antibiotics used in the management or/and treatment of *Salmonella* infection in Ghana. Of the 101 *Salmonella* bacteria isolated, 34 (33.7%) were classified as MDR out of which 23 (67.6%) and 11 (32.4%) were MDR S. Typhi and NTS, respectively. This is consistent with high rates of MDR *S. Typhi* reported in India (97%), Cambodia (90%), Iraq (83%), Nigeria (34%) and Egypt (36%) and NTS reported in Cambodian (33%) and Ugandan (15.4%) populations [**26-30**]. The impact of MDR is very devastating; leading to increased morbidity and mortality, especially in children under age 5 years (as seen in this study) and those who are malnourished [**31**]. This study adds to the body of evidence that mass immunization (in the case of typhoid fever) in endemic areas, rational use of antibiotics, improvement in public sanitary conditions, promotion of safe drinking water and food handling practices are still lacking [**32**]. The rate of ciprofloxacin (a fluoroquinolone) and other cephalosporins resistance in both *S. Typhi* and NTS remained low, similar to previous studies conducted in Ghana [**13**, **22**, **33**, **34**] but significantly higher in other countries [**35**, **36**] including Democratic Republic of Congo [**37**] and Kenya [**38**]. In Ghana, prices of ciprofloxacin and ceftriaxone are expensive; limiting their widespread use and that might explain why resistance to these drugs is still low. To better understand how resistant bacterial strains emerge and spread, it is important to know the molecular basis of their resistance and how the resistant determinants (usually plasmids) are characterized. The action(s) of plasmids are regulated by their replication controls (replicons) and these have proven to be an effective way to characterize them into their incompatibility groups [**11**].

More often than not, resistance to ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole and tetracycline (AMPCSXTTET) are heavily encoded on plasmids with incompatibility group *Inc* HI1 [**7**, **9**, **39**]. These agree partly with our study as 6 out of 11 MDR *S. Typhi* isolates that belonged to *Inc*HI1 produced resistance pattern AMPCSXTTET. However, 5 other MDR isolates of *Inc*HI1 yielded different antibiotic resistance patterns (**Table 6**). Again, we identified MDR *Salmonella* strains belonging to other incompatibility groups (*Inc*FIIS and *Inc*U) conferring resistance with pattern AMPCSXTTET that had never been reported in Ghana and the rest of Africa. This indicates that plasmids belonging to incompatibility group *Inc*HI1 is not the sole source of resistance to ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole and tetracycline as previously thought. Plasmids with incompatibility group *Inc*F encountered in clinical bacteria strains are mostly associated with the spread of antimicrobial resistance and virulence genes [**40**], key among them include the $bla_{CTX\text{-}M\text{-}15}$ and *Inc*FIme plasmids, respectively [**17**]. Characterisation of some bacteria strains, especially *Salmonella enterica, Shigella* sp., enterohaemorrhagic *E. coli* (EHEC),

enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC) by their virulence traits such as bacteriocins, siderophores, adhesion factors and cytotoxins are specifically encoded on *Inc*F plasmids [**40**]. A subtype of *Inc*F plasmid identified in this study, *Inc*FIIS, is responsible for carrying transfer, MDR and virulence functions [**40**, **41**]. The most predominant replicon detected in this study belonged to the incompatibility group *Inc*U, similar to a study reported in Brazil [**42**]. Plasmids in this group are commonly isolated from many clinical and environmental strains of *Escherichia coli* and *Aeromonas* sp. with a wide global distribution [**43**, **44**]. These plasmid replicons have a broad-host-range and are capable of carrying transfer and multidrug-resistance functions [**40**, **45**]. Aside *Inc*U plasmids encoding MDR in this study, other studies have reported that many *Inc*U plasmids encode plasmid-mediated quinolone resistance determinants: *qnr*S2 and aac(6')-Ib-cr [**42**, **46**, **47**]. A variant of narrow-host-range *Inc*X plasmid we identified, *Inc*X2, is a rare plasmid type commonly found in Enterobacteriaceae [**48**]. *Inc*X2 plasmids are receiving much attention lately as they harbor and transfer carbapenem and colistin resistant genes such as bla_{NDM}, bla_{KPC} and *mcr-1* [**49**]. Furthermore, a study in Nigeria shows that *Inc*X plasmids serve as viable platform for dissemination of plasmid-mediated quinolone resistance (*qnr*) genes, especially *qnr*S1 [**50**]. It is worthy of note that IncHI1 and *Inc*U plasmids encoding MDR serovar Typhi were more prevalent in male children within the 6-10 years age group, while *Inc*FIIS and *Inc*X2 found in MDR NTS were predominantly found in female children less than 1 to 5 years. However, it remains unclear why male children infected with MDR S. Typhi had high preponderance to *Inc*HI1 and *Inc*U whereas female children had high affinity to *Inc*X2 and *Inc*FIIS plasmid variants, although they were statistically not significant. This could suggest that genotypic distribution of both MDR Typhi and NTS might possibly be age and gender dependent, which could be beneficial for vaccination programmes that targets these age groups. Further, the likelihood that an MDR infection from rural Ghana could possess plasmid with incompatibility group HI1 is high compared to those living in urban.

Conclusion

This study reports on plasmid variants in two study populations in Ghana that might possibly confer multiple drug-resistance in typhoidal and non-typhoidal *Salmonella* serovars from clinical isolates. Some variants are similar to those already known circulating in Africa (*Inc*HI1, *Inc*X2 and *Inc*U) while one is not yet known (*Inc*FIIS). Further studies should look into using a more rigorous technique such as multi-locus sequence typing (MLST) and/or whole genome sequencing to characterize plasmids associated with MDR to provide more comprehensive data on plasmid-mediated MDRs across the regions in Ghana and Africa.

What is known about this topic

- Multidrug-resistant (MDR) *Salmonella enterica* infection in humans causes significant economic burden on patient as a result of treatment failure and subsequently death;
- These MDR *Salmonella* strains are largely encoded on conjugative plasmids.

What this study adds

- Cases of invasive MDR typhoidal and non-typhoidal *Salmonella* are still alarming;
- The use of molecular typing technique to classify plasmids possibly encoding MDR *Salmonella* has revealed 4 plasmid variants circulating within the study sites in Ghana;
- To strengthen health systems, antimicrobial resistance surveillance and distribution of MDR plasmids should be given attention, and this information could help direct interventional measures to where they are needed most and advocate for a strong surveillance system to be put in place to strengthen and monitor the progressive diffusion of plasmid-mediated MDR *Salmonella* strains in Ghana and Africa at large.

Competing interests

The authors declare no competing interests.

Authors´ contributions

Conception and designing the experiments: Ellis Owusu-Dabo, Michael Owusul, Godfred Acheampong, and Florian Marks. Performing the experiments: Michael Owusul & Godfred Acheampong. Data collection and analysis: Michael Owusul, Godfred Acheampong, and Isaac Osei. Contributing reagents/materials/analysis tools: Ellis Owusu-Dabo Michael Owusul and Se Eun Park. Writing the paper: Godfred Acheampong & Michael Owusul. Critical review of the manuscript: Alex Owusu-Ofori, Isaac Osei, Nimako Sarpong Ellis Owusu-Dabo Michael Owusul.

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Tables and figure

References

1. WHO. ***Salmonella* (Non-typhoidal)**. 2017. Accessed on October 8, 2017.
2. Eng SK, Pusparajah P, Ab Mutalib NS, Ser HL, Chan KG, Lee, LH. *Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science. 2015; 8(3):284-293. **Google Scholar**
3. Gal-Mor O, Boyle EC, Grassl GA. Same species, different diseases: how and why typhoidal and non-typhoidal Salmonella enterica serovars differ. Frontiers in microbiology. 2014;5:391. **PubMed** | **Google Scholar**
4. Andoh LA, Ahmed S, Olsen JE, Obiri-Danso K, Newman MJ, Opintan JA *et al*. Prevalence and characterization of *Salmonella* among humans in Ghana. Tropical medicine and health. 2017;45:3. **PubMed** | **Google Scholar**
5. Al-Emran HM, Krumkamp R, Dekker DM, Eibach D, Aaby P, Adu-Sarkodie Y *et al*. Validation and identification of invasive Salmonella serotypes in Sub-Saharan Africa by multiplex polymerase chain reaction. Clinical Infectious Diseases. 2016;62(suppl1):S80-S82. **PubMed** | **Google Scholar**
6. Espié E, De Valk H, Vaillant V, Quelquejeu N, Le Querrec F, Weill F. An outbreak of multidrug-resistant Salmonella enterica serotype Newport infections linked to the consumption of imported horse meat in France. Epidemiology & Infection. 2005 April 8;133(2):373-376. **PubMed** | **Google Scholar**
7. Mills-Robertson F, Addy ME, Mensah P, Crupper SS. Molecular characterization of antibiotic resistance in clinical Salmonella typhi isolated in Ghana. FEMS microbiology letters. 2002;215(2):249-253. **PubMed** | **Google Scholar**
8. Mills-Robertson F, Crupper S, Addy M, Mensah P. Antibiotic resistance and genotyping of clinical group B Salmonella isolated in Accra, Ghana. Journal of applied microbiology. 2003 Jan 13;94(2):289-294. **PubMed** | **Google Scholar**
9. Shanahan PM, Jesudason MV, Thomson CJ, Amyes SG. Molecular analysis of and identification of antibiotic resistance genes in clinical isolates of Salmonella typhi from India. Journal of Clinical microbiology. 1998 June; 36(6):1595-1600. **PubMed** | **Google Scholar**
10. Francia MV, Varsaki A, Garcillán-Barcia MP, Latorre A, Drainas C, de la Cruz F. A classification scheme for mobilization regions of bacterial plasmids. FEMS microbiology reviews. 2004 Feb; 28(1):79-100. **PubMed** | **Google Scholar**
11. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. Journal of microbiological methods. 2005 June 2;63(3):219-228. **PubMed** | **Google Scholar**
12. Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C *et al*. Plasmid replicon typing of commensal and pathogenic Escherichia coli isolates. Applied and environmental microbiology. 2007;73(6):1976-1983. **PubMed** | **Google Scholar**
13. Labi A-K, Obeng-Nkrumah N, Addison NO, Donkor ES. Salmonella blood stream infections in a tertiary care setting in Ghana. BMC infectious diseases. 2014;14:3857. **PubMed** | **Google Scholar**
14. Feglo P, Frimpong E, Essel-Ahun M. Salmonellae carrier status of food vendors in Kumasi, Ghana. East African medical journal. 2004 July;81(7):358-361. **PubMed** | **Google Scholar**
15. Gravel J, Ceroni, D, Lacroix, L, Renaud, C, Grimard, G, Samara, E *et al*. Association between oropharyngeal carriage of *Kingella kingae* and osteoarticular infection in young children: a case–control study. Canadian Medical Association Journal. 2017;189(35):E1107-E1111. **PubMed** | **Google Scholar**

16. CLSI. **Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. CLSI standards for antimicrobial susceptibility testing** . 2015. Accessed on October 8, 2017
17. Villa L, García-Fernández A, Fortini D, Carattoli A. Replicon sequence typing of *Inc*F plasmids carrying virulence and resistance determinants. Journal of antimicrobial chemotherapy. 2010;65(12):2518-2529. **PubMed** | **Google Scholar**
18. García-Fernández A, Villa L, Carta C, Venditti C, Giordano A, Venditti M *et al*. Klebsiella pneumoniae ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrobial agents and chemotherapy. 2012 Apr;56(4):2143-5. **PubMed** | **Google Scholar**
19. Buckle GC, Walker C, Black RE. Typhoid fever and paratyphoid fever: Systematic review to estimate global morbidity and mortality for 2010. J Glob Health. 2012;2(1):10401. **PubMed** | **Google Scholar**
20. Gill CJ, Hamer DH. Foodborne illnesses. Current treatment options in gastroenterology. 2001;4(1):23-38. **PubMed** | **Google Scholar**
21. Gilchrist JJ, MacLennan CA, Hill AV. Genetic susceptibility to invasive Salmonella disease. Nature reviews Immunology. 2015;15(7):452-63. **PubMed** | **Google Scholar**
22. Nielsen MV, Sarpong N, Krumkamp R, Dekker D, Loag W, Amemasor S *et al*. Incidence and characteristics of bacteremia among children in rural Ghana. PloS one. 2012;7(9):e44063. **PubMed** | **Google Scholar**
23. Laxminarayan R, Chaudhury RR. Antibiotic resistance in India: drivers and opportunities for action. PLoS medicine. 2016;13(3):e1001974. **PubMed** | **Google Scholar**
24. Lee K, Yong D, Yum, JH, Lim YS, Kim HS, Lee BK *et al*. Emergence of multidrug-resistant Salmonella enterica serovar typhi in Korea. Antimicrobial agents and chemotherapy. 2004; 48(11):4130-4135. **PubMed** | **Google Scholar**
25. Yoon HJ, Cho SH and Kim SH. A case of multidrug-resistant Salmonella enterica serovar typhi treated with a bench to bedside approach. Yonsei medical journal. 2009;50(1):147-151. **PubMed** | **Google Scholar**
26. Balaji V, Sharma A, Ranjan P, Kapil A. Revised ciprofloxacin breakpoints for Salmonella Typhi: its implications in India. Indian journal of medical microbiology. 2014;32(2):161. **PubMed** | **Google Scholar**
27. Rahman B, Wasfy M, Maksoud M, Hanna N, Dueger E, House B. Multi-drug resistance and reduced susceptibility to ciprofloxacin among Salmonella enterica serovar Typhi isolates from the Middle East and Central Asia. New microbes and new infections. 2014;2(4):88-92. **PubMed** | **Google Scholar**
28. Vlieghe ER, Phe T, De Smet B, Veng CH, Kham C, Bertrand S, Vanhoof R *et al*. Azithromycin and ciprofloxacin resistance in Salmonella bloodstream infections in Cambodian adults. PLoS neglected tropical diseases. 2012;6(12):e1933. **PubMed** | **Google Scholar**
29. Mathew B, Ugboko H, De N. Prevalence of Multidrug Resistant Salmonella enterica Serovar Typhi in Kaduna Metropolis, Kaduna, Nigeria. International Journal of Current Microbiology and Applied Sciences. 2015;4(9):323-335. **Google Scholar**
30. Odoch T, Wasteson Y, L'Abée-Lund T, Muwonge A, Kankya C, Nyakarahuka L *et al*. Prevalence, antimicrobial susceptibility and risk factors associated with non-typhoidal Salmonella on Ugandan layer hen farms. BMC veterinary research. 2017;13(1):365. **PubMed** | **Google Scholar**
31. Kumar R, Gupta N, Shalini. Multidrug-resistant typhoid fever. Indian J Pediatr. 2007;74(1):39-42. **PubMed** | **Google Scholar**

32. Zaki SA, Karande S. Multidrug-resistant typhoid fever: a review. The Journal of Infection in Developing Countries. 2011;5(5):324-337. **PubMed** | **Google Scholar**

33. Marks F, Adu-Sarkodie Y, Hünger F, Sarpong N, Ekuban S, Agyekum A *et al*. Typhoid fever among children, Ghana. Emerg Infect Dis. 2010;16(11):176-78. **PubMed** | **Google Scholar**

34. Groß U, Amuzu SK, Ciman RD, Kassimova I, Groß L, Rabsch W *et al*. Bacteremia and antimicrobial drug resistance over time, Ghana. Emerg Infect Dis. 2011 Oct;17(10):1879–1882. **PubMed** | **Google Scholar**

35. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK *et al*. Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio. 2018;9(1):e00105-18. **PubMed** | **Google Scholar**

36. Yan JJ, Chiou CS, Lauderdale TL, Tsai SH, Wu JJ. Cephalosporin and ciprofloxacin resistance in Salmonella, Taiwan. Emerging infectious diseases. 2005;11(6):947. **PubMed** | **Google Scholar**

37. Phoba MF, Barbé B, Lunguya O, Masendu L, Lulengwa D, Dougan G. Salmonella enterica serovar Typhi producing CTX-M-15 extended spectrum ß-lactamase in the Democratic Republic of the Congo. Clinical Infectious Diseases. 2017;65(7):1229-1231. **PubMed** | **Google Scholar**

38. Kariuki S, Revathi G, KiiruJ, Mengo D.M, Mwituria J, Muyodi J *et al*. Typhoid in Kenya is associated with a dominant multidrug-resistant Salmonella enterica serovar Typhi haplotype that is also widespread in Southeast Asia. Journal of clinical microbiology. 2010;48(6):2171-2176. **PubMed** | **Google Scholar**

39. Rowe B, Ward LR, Threlfall EJ. Multidrug-resistant Salmonella typhi: a worldwide epidemic. Clinical Infectious Diseases. 1997;24(Suppl_1):S106-S109. **PubMed** | **Google Scholar**

40. Johnson TJ, Nolan LK. Pathogenomics of the virulence plasmids of Escherichia coli. Microbiology and Molecular Biology Reviews. 2009;73(4):750-774. **PubMed** | **Google Scholar**

41. Bielak EM. Diversity and epidemiology of plasmids from Enterobacteriaceae from human and non-human reservoirs. Kgs Lyngby: Technical University of Denmark (DTU), 2012: 188. **Google Scholar**

42. Seribelli AA, Ballaben AS, Ferreira JC, Medeiros MIC, Darini ALC, Falcao JP. *Inc*U plasmid harbouring blaCTX-M-8 in multidrug-resistant Shigella sonnei in Brazil. Journal of global antimicrobial resistance. 2018;14:99-100. **PubMed** | **Google Scholar**

43. Piotrowska M, Popowska M. Insight into the mobilome of Aeromonas strains. Frontiers in microbiology. 2015;6:494. **PubMed** | **Google Scholar**

44. Marti E, Balcázar JL. Multidrug resistance-encoding plasmid from Aeromonas sp. strain P2G1. Clinical Microbiology and Infection. 2012;18(9):E366-E368. **PubMed** | **Google Scholar**

45. Dang B, Xu Y, Mao D, Luo Y. Complete nucleotide sequence of plasmid pNA6 reveals the high plasticity of *Inc*U family plasmids. Gene. 2016; 591(1):74-79. **PubMed** | **Google Scholar**

46. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy. 2005; 56(3):463-469. **Google Scholar**

47. Carattoli A. Plasmids and the spread of resistance. International Journal of Medical Microbiology. 2013; 303(6-7):298-304. **PubMed** | **Google Scholar**

48. Fortini D, Fashae K, Villa L, Feudi C, García-Fernández A, Carattoli A. A novel plasmid carrying blaCTX-M-15 identified in commensal Escherichia coli from healthy pregnant

women in Ibadan, Nigeria. Journal of global antimicrobial resistance. 2015;3(1):9-12. **PubMed** | **Google Scholar**

49. Guo Q, Su J, McElheny CL, Stoesser N, Doi Y, Wang M. *Inc*X2 and *Inc*X1-X2 hybrid plasmids coexisting in FosA6-producing Escherichia coli. Antimicrobial agents and chemotherapy. 2017;61(7) pii: e00536-17. **PubMed** | **Google Scholar**
50. Sumrall ET, Gallo EB, Aboderin AO, Lamikanra A, Okeke IN. Dissemination of the transmissible quinolone-resistance gene qnrS1 by *Inc*X plasmids in Nigeria. PloS one. 2014;9(10):e110279. **PubMed** | **Google Scholar**