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# Plasmid profiling and incompatibility grouping of multidrug resistant *Salmonella enterica* serovar Typhi isolates in Nairobi, Kenya

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## Abstract

**Objectives:** Plasmids harbour antibiotic resistance genes which contribute to the emergence of multidrug resistant pathogens. We detected the presence of plasmids in multidrug resistant *Salmonella enterica* serovar Typhi (*S. Typhi*) isolates from our previous study and consequently determined their incompatibility groups and possibility of conjugation transmission. Plasmids were extracted from 98 multidrug resistant *S. Typhi* isolates based on alkaline lysis technique. Plasmid incompatibility grouping was established by PCR replicon typing using 18 pairs of primers to amplify FIA, FIB, FIC, HI1, HI2, I1-Iy, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA replicons. Antibiotic resistance phenotypes were conjugally transferred from *S. Typhi* isolates with plasmids to *Escherichia coli* K12F strain devoid of plasmids.

**Results:** Approximately 79.6% of the MDR *S. Typhi* isolates were related to the existence of plasmids. We detected 93.6% of plasmids belonging to incompatibility (Inc) group HI1. The other incompatibility groups identified included IncFIC (16.7%), IncP (1.3%), and IncI1 (1.3%) which appeared together with Inc HI1. MDR *S. Typhi* isolated carried a homologous plasmid of incompatibility group HI1 most of which transferred the resistance phenotypes of ampicillin, tetracycline and chloramphenicol to the transconjugants.

**Keywords:** Multidrug resistant *S. Typhi*, Plasmid incompatibility grouping, Conjugation

## Introduction

Plasmids have been defined as circular double-stranded deoxyribonucleic acid (DNA) molecules that exist and replicate independently within a bacterial cell or can be integrated into the bacterial chromosome. Plasmids serve as vectors for lateral mobility of genetic information between bacterial cell consequently promoting their spread and sustainability within a bacterial niche under changing environmental stimuli [1]. Genes encoding different traits in a bacteria are lugged in plasmids and are expressed when the bacteria encounters a contentious environment especially those inflicted by human activities including antibiotic use [2]. This phenomenon has played a big role in evolution of antibiotic resistance bacteria especially among gram-negative Enterobacteriaceae

contributing to treatment failure and persistence of infectious diseases in the population [3, 4].

Plasmids classification based on their stability during transmission is evaluated by exploring their incompatibility categories. Incompatibility grouping represents “the inability of two plasmids to coexist stably over a number of generations in the same bacterial cell line” such that plasmids incompatible to each other are assigned the same group [5]. This mechanism was first described in the early 1970s by Datta and Hedges and about 30 incompatibility groups of plasmids have so far been recognized in the family of Enterobacteriaceae [6]. Further, based on genetic relatedness the incompatibility groups have been sub-grouped into four major clusters: IncF group (IncF, IncS, IncC, IncD, IncJ); IncP group (IncP, IncU, IncM, IncW); Ti group (IncX, IncH, IncN, IncT) and IncI group (IncI, IncB and IncK) [7]. Most of these plasmids especially those of incompatibility groups IncA/C, B/O, L/M, HI1, HI2, I1, N, F, and P are associated with multidrug resistant (MDR) isolates from clinical, animal and

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environmental samples [4, 8–10]. MDR *S. Typhi* mostly harbors plasmid of IncHI, however other incompatibility groups including IncF, IncP, and IncB/O plasmids have also been identified [11, 12].

With the rapidly evolving molecular techniques, more advanced plasmid analysis tools with a high discriminatory power and a quick turnaround time are constantly being developed. These tools provide more precise results for epidemiological investigations. However in settings where these tools are not accessible simple molecular techniques including plasmid replicon typing still provides an insight on the role of plasmids in transmitting antibiotic resistance. Therefore in this work we detected the existence of plasmids in previously isolated MDR *S. Typhi* strains [13] and analysed the plasmids by means of incompatibility grouping adopting a PCR based replicon typing protocol illustrated by Carattoli et al. [14]. We further screened the plasmids for their ability to transfer antibiotic resistance to an *E. coli* strain short of plasmids.

## Main text

### Methods

#### Plasmid extraction

The 98 archived MDR *S. Typhi* isolates from our previous study [13] were sub-cultured onto MacConkey agar. Discrete colonies were then transferred into 3 ml of Luria broth and incubated at 37 °C overnight on a shaker (250 rpm). After 18 h incubation, liquid culture was transferred to a 1.5 ml eppendorf tube and centrifuged to harvest the cells at the bottom of the tube. Plasmids were extracted from the sediment cell pellet using alkaline lysis protocol described by Birnboim and Doly [15]. *E. coli* strain 39R861 (NCTC 50192) with known plasmids of molecular size of 98, 42, 23.9 and 4.6 MDa size markers was used as the control strain. The products were analysed on 1.5% agarose gel.

#### PCR plasmid incompatibility grouping

Plasmid incompatibility grouping was based on the method previously described by Carattoli et al. [14]. Briefly, 18 pairs of primers targeting replicons FIA, FIB, FIC, HI1, HI2, I1, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA were used for conventional amplification in a five multiplex- and three simplex-PCR regimen. The amplicons were visualized under UV transilluminator in an electrophoresis stained with ethidium bromide. The incompatibility groups were identified based on the amplicon sizes described by Carattoli et al. [14].

#### Transferable resistance plasmids

In-vitro conjugation experiment on transferable plasmids were performed according to the method described by Walia et al. [16], *E. coli* K12F devoid of plasmids

and resistant to nalidixic acid was used as the recipient organism for this experiment. From the MDR *S. Typhi* isolates we selected 68 isolates resistant to ampicillin and sensitive to nalidixic acid. The transconjugants were then screened on MacConkey agar plates integrated with nalidixic acid (32 mg/l) and ampicillin (32 mg/l). To determine the transferable resistance-encoding plasmids, plasmid DNA were extracted from the transconjugants and visualised on a 1% agarose gel electrophoresis.

## Results

### Plasmid analysis

Approximately 79.6% of the 98 MDR *S. Typhi* isolates expressed a larger 98 MDa plasmid and 54% a smaller 42 MDa plasmid as shown in Table 1 and Fig. 1 (lanes 1–7). IncHI1 plasmid predominated among the replicon types identified (73/78, 93.6%) as represented in Table 1 and Fig. 2a, the other replicon types observed included IncFIC (13/78, 16.7%) (Fig. 2b), IncI1 (1/78, 1.3%) (Fig. 2c) and IncP (1/78, 1.3%) (Fig. 2d). Three sets of combined replicon types were observed IncHI1 + IncFIC (11/78, 14.1%), IncHI1 + IncFIC + IncP (1/78, 1.3%) and IncHI1 + IncFIC + IncI (1/78, 1.3%) as shown in Table 1. Plasmids and the replicon types prevailed among the isolates that exhibited resistance to tetracycline, chloramphenicol and ampicillin. Interestingly, we also observed the reduction of IncHI1 replicon types during the study period (Table 1).

### Conjugation experiments

Transconjugants grew on plates containing both ampicillin (32 mg/l) and nalidixic acid (32 mg/l). Of the 68 MDR *S. Typhi* isolates selected for conjugation, 45 (66.2%) isolates transferred resistance of one or more antimicrobials to recipient *E. coli* K12 (Table 1). Twenty-eight (41.2%) isolated transferred full resistance phenotype with majority transfer being that of tetracycline, chloramphenicol and ampicillin (23/28, 82.1%). Additionally 23 (33.8%) isolates did not transfer any of the resistant traits to the recipient. The acquisition of the antibiotic resistance phenotypes in the transconjugants was related to the presence of 98 MDa size plasmids and in some isolates a smaller plasmid of 42 MDa (Additional file 1: Fig. S1; Additional file 2: Fig. S2).

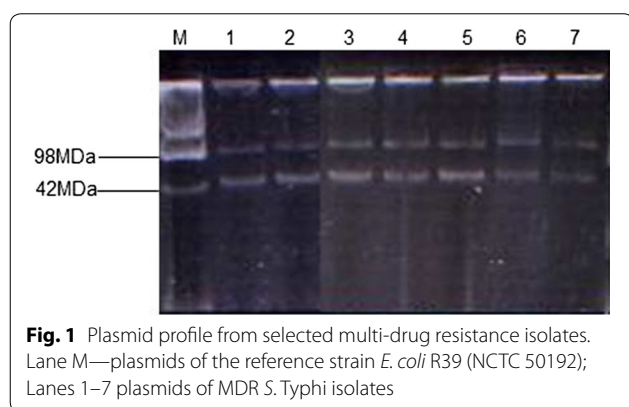
## Discussion

In this current study 79.6% of the MDR *S. Typhi* carried large self-transmissible plasmids of >98 MDa. Additionally the main replicon identified among the plasmids analysed were those of incompatibility group HI1. Plasmid of this incompatibility group could be accounting for the persistence and spread of MDR *S. Typhi* isolates. Since the first report of an IncHI1 plasmid

**Table 1 Summary of the distribution of plasmid sizes, incompatibility groups and transconjugants in relation to the resistance phenotypes identified**

Resistance phenotype	Plasmid size 42 MDa N (%)	Plasmid size 98 MDa N (%)	Incompatibility groups (N)			Resistance pattern of transconjugants (N)
			2004	2005	2006	
AMC,TET,C,AMP (N = 10)	1 (1.0%)	4 (4.1%)	HI(4)	–	–	AMC,TET,C,AMP(5) TET,C,AMP(1) AMC,AMP(1) TET(1) NT(2)
CF,TET,C,AMP (N = 2)	0 (0.0%)	2 (2.0%)	HI(1)	HI(1)	–	TET,C,AMP(1) TET,AMP (1) NT(1)
TET,C,AMP (N = 71)	52 (53.0%)	70 (71.4%)	HI(25), FIC(6), I(1)	HI(24), FIC(4), P(1)	HI(17), FIC(3)	TET,C,AMP(23) TET,AMP(4) C,AMP(1) TET(4) C(2) AMP(1) NT(21)
Others (N = 11)	0 (0.0%)	2 (2.0%)	HI(1)	–	–	–

AMP ampicillin, AMC amoxicillin–clavulanic acid, CF ciprofloxacin, C chloramphenicol, TET tetracycline, NT not transferable



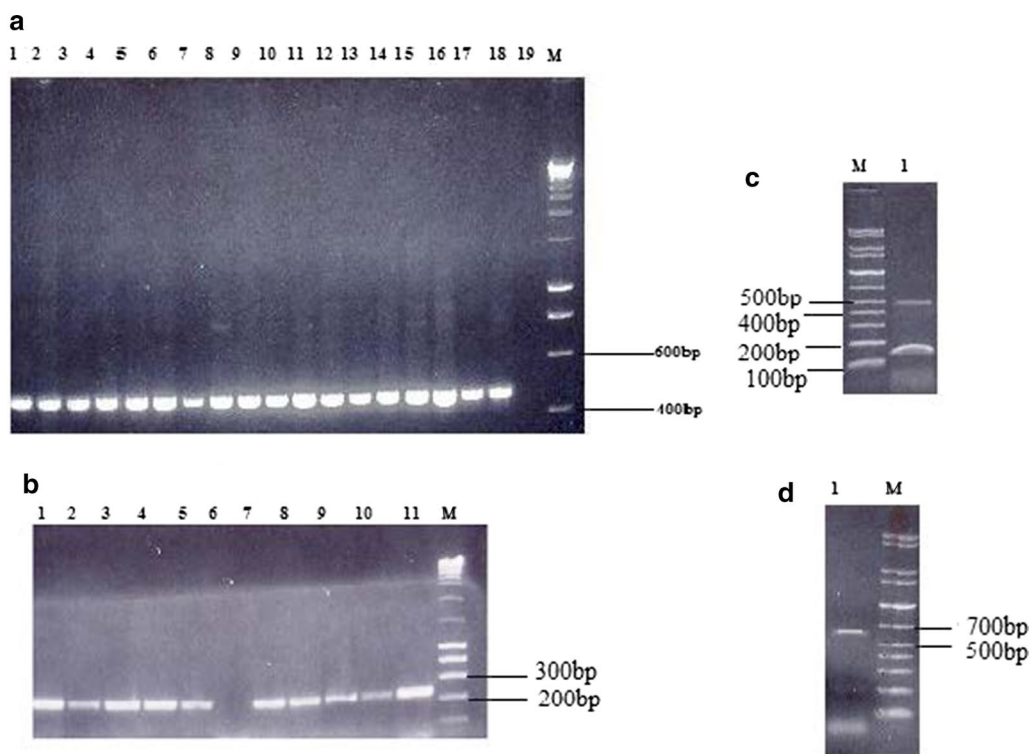
encoding MDR *S. Typhi* isolates in Mexico City in 1972, many studies globally are currently reporting similar isolates among MDR *S. Typhi* and other Enterobacteriaceae [17–22].

The most common resistance phenotypes among the isolates in this study were linked to tetracycline, ampicillin and chloramphenicol resistance and were significantly associated with the presence of plasmids mainly of IncHI1. Plasmids of incompatibility group HI1 (IncHI1) have been shown to encode multiple antibiotic resistances specifically to ampicillin, chloramphenicol, trimethoprim, sulfonamides, streptomycin and tetracyclines and most of which are associated with outbreaks [17, 23, 24]. The results from the current study show that it is likely that the same plasmid of IncHI1 could be circulating and spreading widely within the MDR

strains of *S. Typhi* however, this would have been well supported by comparative analysis to detect the clustering of these plasmids.

These homologous plasmids among the isolates analysed may have been acquired from other *Salmonella enterica* strains or from other Enterobacteriaceae as has been previously investigated that IncHI1 plasmid can be transferred between the Enterobacteriaceae or naturally through horizontal gene transfer among *S. Typhi* strains [25–27]. More studies based on sequence analysis of the conjugative plasmids reveal that these plasmids are closely related and share related resistance genes suggesting that this plasmids that confer antibiotic resistance may have been transferred between these serovars [28, 29]. It is unfortunate that this study did not go further to perform plasmid sequence to prove this hypothesis.

Other than IncHI1 we further identified other replicons including IncI1, IncP, and IncFIC all of which co-existed together with Inc HI1. Plasmids belonging to the IncI1 family are considered as virulence plasmids encoding type IV pili that play a role in attachment of the bacteria to the host cells. However in this context IncI1 plasmids have been described in *E. coli*, *Salmonella* and other Enterobacteriaceae as vectors of resistant genes that code for extended-spectrum  $\beta$ -lactamase (ESBL) predominantly *bla*<sub>CTX-M</sub>, *bla*<sub>TEM-1</sub> and *bla*<sub>SHV</sub> and to a lesser extent plasmid mediated AmpC  $\beta$ -lactamase gene (*bla*<sub>CMY-2</sub>) that code for cephamycin and carbapenem resistance [30–32]. IncP plasmids on the other hand are widely distributed in the environment habitat including water, soil and waste water treatment plants among



**Fig. 2** Gel electrophoresis showing the amplicon products of Incompatibility groups IncHI1, IncFIC, IncI1 and IncP. **a** Lanes 1–18—471-bp IncHI1 amplicon, lane 19—*S. Typhi* sensitive to all the antibiotics tested, lane M—200 bp MW ladder; **b** Lane 1, 2, 3, 4, 5, 7, 8, 9, 10, 11—IncFIC (262 bp), lane 6 MDR *S. Typhi* without IncFIC; **c** Lane 1—IncI1 (139 bp); **d** Lane 1—IncP (534 bp) lane M 100 bp marker

Enterobacteriaceae family and carry genes that confer antibiotics and heavy metal resistance [33–37]. Recently, *Salmonella enterica* serovar Typhimurium strain resistant to colistin and carried by an IncP plasmid was isolated from a healthy individual. Additionally conjugation experiments from this same study indicated that the plasmids played a role in dissemination of *mcr-1* gene responsible for colistin resistance indicating that the individual may have been a carrier facilitating the spread of resistant strains [38].

Conjugation experiment indicated that 66.2% of the MDR selected for conjugation transferred some or all of the resistance phenotypes to *E. coli* K12. It is certain that the conjugal transfer of antibiotic resistance to the transconjugants may have occurred via plasmids consequently impelling the spread of antibiotic resistance.

Antibiotic resistance in MDR *S. Typhi* mainly to first line antibiotics is encoded by plasmids. In this study we show that conjugative plasmids especially those of IncHI1 were relevant in disseminating antibiotic resistance predominantly the first line antibiotics. Knowing the predominant replicon type forms a basis for future studies with approaches intended to construct genes that block horizontal transfer of this group of plasmids which have

been successfully utilized to counter other incompatibility group of plasmids [39, 40].

### Limitations

The information gathered in this study affirms the role of plasmids in driving antibiotic resistance however comparative genomic analysis addressing the evolution of these plasmids categorically how the loss, gain and persistence of certain groups of plasmids facilitates the dissemination of antibiotic resistance bacterial pathogens would have supported this study.

### Additional files

**Additional file 1: Fig. S1.** Plasmids extracted from the transconjugants. Lane M plasmids of *E. coli* 39 (NCTC 50192); Lane 1–10 transconjugants resistant to chloramphenicol, tetracycline and ampicillin; Lane 5 non-conjugative transconjugant.

**Additional file 2: Fig. S2.** MacConkey culture plates showing the morphology of the recipient, donor and the transconjugant. (A): Colonies of the recipient strain (*E. coli* K12) in presence of nalidixic acid; (B): Colonies of the donor strain (*S. Typhi*) on culture plate containing ampicillin; (C): Colonies of transconjugants on culture plate containing both ampicillin and nalidixic acid.

## Abbreviations

MDR: multidrug resistant; Inc: incompatibility group; NCTC: National Collection of Type Cultures; ESBL: extended-spectrum  $\beta$ -lactamase resistance; MDa: megadalton.

## Acknowledgements

Not applicable.

## Authors' contributions

WM, PW, SK, and AM designed the study. WM performed the laboratory investigations, analysed the data and prepared the manuscript. All authors read and approved the final manuscript.

## Funding

No funding was received.

## Availability of data and materials

The datasets analysed in this study are provided as supporting documents.

## Ethics approval and consent to participate

This study was approved by Kenya Medical Research Institute (KEMRI) Scientific Steering Committee and assigned SSC No. 1320.

## Consent to publish

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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Received: 13 May 2019 Accepted: 11 July 2019

Published online: 16 July 2019

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