

Original Research Article

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## Detection of the *tet A* Resistance Gene in Broad Spectrum Beta-lactamase (ESBL)-Producing *Escherichia coli* Strains Isolated from Faecal Waste of Pigs on a Farm in Abidjan, Côte d'Ivoire

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### ABSTRACT

The aim of the study was to detect the *tet A* gene coding for tetracycline resistance in ESBL-producing strains of *Escherichia coli* isolated from faecal waste from pigs used as manure. Thirty-five *E. coli* strains containing genes coding for ESBL resistance were used in the study. These strains were isolated from faecal waste from pigs used to produce manure. They were identified using biochemical tests. An antibiotic susceptibility test was carried out using the agar diffusion antibiogram technique to determine antibiotic resistance profiles and confirmed the presence of ESBL production. PCR was carried out to search for the *tet A* gene coding for tetracycline resistance in strains likely to produce ESBL. Antibiogram results of the 35 *E. coli* strains confirmed the ESBL phenotype. These strains were resistant to several antibiotics. The rate of resistance to tetracycline was 100%. All these strains harboured the *tet A* resistance gene. They also presented five genetic combinations, the most predominant of which was *bla*<sub>TEM</sub>, *tet A* with a frequency of 38%. The detection of *tet A* resistance genes in *E. coli* represents a risk to public health. Resistance genes circulating in the digestive flora of farm animals therefore need to be monitored to prevent their spread.

#### Keywords

*tet A* resistance gene, Tetracycline, Faecal waste, Manure, *E. coli*

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## Introduction

The agricultural sector, particularly livestock farming, is most often called upon by public policies to meet these needs. Indeed, whether formal or informal, livestock farming is a genuine means of improving people's living conditions (Gomgnimbou *et al.*, 2014). The importance of this activity in Côte d'Ivoire, particularly in the peri-urban areas of Abidjan, remains considerable. This is because of its contribution to improving food security, the adoption of animal traction and the increase in soil fertility through the use of manure produced from faeces as a biofertiliser (Blanchard, 2010). Although livestock farming has development implications, it also raises many concerns about the environment and its impact on health (FAO, 2006). Manure management and livestock rearing close to homes is one of the main issues (Manyi-Loh *et al.*, 2016). Through husbandry, storage and spreading practices, manure tends to concentrate numerous components likely to cause disease in both humans and animals. Whether microbes, bad odours, water and soil pollution factors, greenhouse gases, poor manure management poses a real threat to public health (Bagalwa *et al.*, 2013). This is reflected in an increase in the incidence of certain digestive diseases (Mali.MEADR, 2015).

Untreated manure used as fertiliser by market gardeners to improve their crop yields contains high numbers of potentially pathogenic bacteria (Hutchison *et al.*, 2004). These pathogenic bacteria include *E. coli* that are multi-resistant to antibiotics, commensal bacteria in the digestive tract of farm animals that can transfer resistance genes to bacteria in the intestinal flora of humans via food and in the environment via hospital effluents and waste water, posing a real risk to public health (Phillips and Casewell, 2004; Sanders *et al.*, 2011). Tetracycline, an antibiotic widely used in animal husbandry in general and in pigs in particular, can prevent and cure infectious diseases and promote daily weight gain (Mc Ewen, 2002). However, the overuse of these antibiotics in livestock farming has led to the emergence of bacteria resistant to tetracycline (Agero and Sandvang, 2005; Stine *et al.*, 2007). The tetracycline resistance genes expressed by efflux proteins most often detected in faecal isolates from pigs are the *tet A* resistance genes (EFSA, 2011; Agero and Aarestrup, 2013). In Côte d'Ivoire, the impact of manure management using animal faecal waste and the possible risks to public health are poorly known and documented. In this respect, farming practices and the management of manure from animal faeces in

general, and pig faeces in particular, represent a real public health risk. To facilitate microbiological control of manure due to the health impact of antibiotic resistance in bacteria isolated from this waste, the aim of this study was to detect the *tet A* resistance gene in *E. coli* isolated from pig faecal waste used as manure.

## Materials and Methods

### Identification of bacterial strains

In order to confirm the identification of ESBL-producing *E. coli* isolates carrying the *bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub> genes previously detected in a previous study, specific biochemical characteristics were investigated. The strains were taken from deep agar plates and subcultured on Mac-Conkey medium and ordinary medium in order to obtain pure cultures after 24 h of incubation at 37°C. Presumptive *E. coli* isolates were identified using a biochemical test. Biochemical identification was carried out by testing for catalase, cytochrome oxidase C, urease and indole, Simmons citrate utilisation, glucose and lactose, gas production, hydrogen sulphide, lysine deaminase (LDA) and lysine decarboxylase (LDC) (Farmer, 1999).

### Antibiotic susceptibility test

Antibiotic susceptibility testing of these identified *E. coli* strains was carried out using the Muller-Hinton agar diffusion method (Bio-rad, Marne-la-coquette, France). Fourteen (14) antibiotic discs (Bio-rad, Marne-la-coquette, France) were used in the study: Amoxicillin (25 µg), Amoxicillin + clavulanic acid (20/10 µg), Ceftriaxone (30 µg), Cefoxitin (30 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), Tetracycline (30 µg), Trimethoprim / Sulfamethoxazole (1.25/23.75 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Kanamycin (30 µg), Streptomycin (10 µg), Colistin (50 µg).

### Detection of the *tet A* gene encoding tetracycline resistance

*E. coli* strains that exhibited extended-spectrum Betalactamase phenotypes following antibiotic susceptibility testing were used to screen for the *tet A* gene encoding tetracycline resistance. Conventional PCR was used to detect *tet A* genes encoding tetracycline resistance. Bacterial DNA was extracted using the heat shock technique. The final volume of the reaction

mixture was 50 µL and comprised 5µL of coloured buffer (5X Green GoTaq®), 5µL of uncoloured buffer (5X Colorless GoTaq®), 30.3 µL of ultrapure water (Nuclease- Free Water, Promega, USA), 3µL of MgCl<sub>2</sub> (25 mM), 0.5 µL of DNTP (10 mM), 0.5 µL of Forward primer (10 mM), 0.5 µL of Reverse primer (10 mM), 0.2 µL of Taq polymerase (GoTaq®, Promega) and 5 µL of DNA to be amplified. The gene *tet A* primer used was Forward *tet A* F-5'TTGTTCTGAAGTGCCAGTAA-3' and Reverse *tet A* R-5'GACGTCGTTTCGAGTGAACAGA-3' with an amplicon size of 370 base pairs (Sunde and Sorum, 2001). The amplification conditions carried out in the thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) are summarised in Table 1. PCR products were analysed by 1.5% agarose electrophoresis (Invitrogen). The reference strain *E. coli* CIP/ RP4 (Institut Pasteur de Paris) carrying the *tet A* gene was used as a positive control in the PCR test.

## Results and Discussion

All 35 isolates selected exhibited biochemical characteristics specific to *E. coli* (Table 2). Resistance rates to the different antibiotics determined during antibiotic susceptibility testing are presented in Table 3. The ESBL phenotype of the 35 strains was confirmed by the assay and the resistance rates were as follows. Resistance to tetracycline and ceftriaxone was 100%, 90% to streptomycin and amoxicillin, 80% to cotrimoxazole, 70% to gentamicin, kanamycin and amoxicillin + clavulanic acid, 20% to ciprofloxacin and nalidixic acid, 10% to chloramphenicol and 0% to colistin. The 35 strains analysed by PCR showed the presence of the *tet A* resistance gene (100%) with bands 370 base pairs in size (Figure 1). The strains harboured a specific combination of genes: *bla*<sub>TEM</sub>, *bla*<sub>CTX-M</sub>, *bla*<sub>SHV</sub>, *tet A*. Five combinations were detected for the same resistance phenotype. The gene combination *bla*<sub>TEM</sub>, *tet A* was the most predominant with a frequency of 38% (Table 4).

During the study, the 35 ESBL-producing *E. coli* strains isolated from the faecal waste of pigs were all resistant to tetracycline and ceftriaxone, with resistance rates of 100%, 90% to amoxicillin and streptomycin, 80% to cotrimoxazole and 70% to gentamicin. This high rate of resistance to tetracycline during the present study could be justified by the abundant use of this antibiotic in our pig farms. The very high rate of resistance to tetracycline and more than five other antibiotics has also been reported by several authors in studies conducted on *E.*

*coli* strains *E. coli* of animal origin throughout the world (Blanco *et al.*, 1997; Muhammad *et al.*, 2009; Karczmarczyk *et al.*, 2011). In France, a study carried out on the effect of antibiotic use on the resistance of *E. coli* of porcine origin on a farm in Nantes also reported very high rates of resistance to tetracycline, which was 99%, similar to that in the present study (Belloc *et al.*, 2005). Other studies have reported a high rate of tetracycline resistance in *E. coli* strains of animal origin, but lower than that determined in the present study. In India, high resistance to tetracycline and cotrimoxazole (88% and 83% respectively) has been reported in commensal isolated from chicken faeces (Balasubramaniam *et al.*, 2014). These results are also in line with other studies carried out in Toulouse, another French city, where resistance to tetracycline was 90%, streptomycin (82%), chloramphenicol (32%), trimethoprim (58%) and sulphonamide (63%) in *E. coli* from piglets treated with ampicillin (Bibbal *et al.*, 2009). Enne *et al.*, (2007) in Great Britain also detected a tetracycline resistance rate of 78%, 30.8% to cotrimoxazole, 37.5% to streptomycin and 25.4% to ampicillin in *E. coli* strains isolated from a cattle and sheep farm. Daini and Adesemowo (2008) in a study in Nigeria reported tetracycline resistance of 88% and gentamicin resistance of 54% in *E. coli* strains of clinical origin. The majority of studies revealing the high prevalence of tetracycline resistance in *E. coli* isolated from faecal waste from pigs and in the present study showed that tetracycline is one of the most widely used antibiotics in the animal world (McEwen, 2002). This is also reported in an epidemiological study on trends in the use of antibiotics in the pig industry, which revealed that tetracycline was the leading antibiotic (Anses-ANMV, 2014). During the study, all 35 ESBL-producing *E. coli* strains showed the presence of the *tet A* gene (100%). This prevalence of the *tet A* gene is sometimes at odds with other results reported by several authors.

In England, a study carried out on a farm reported a 17.5% prevalence of the *tet A* gene in *E. coli* isolated from pigs and sheep (Enne *et al.*, 2007). In India, another study conducted on commensal *E. coli* strains of avian origin reported a 29% prevalence of the *tet A* gene.

Strains that did not show the presence of the *tet A* resistance gene could contain other genes encoding resistance to tetracycline, such as the *tet B*, *tet C* and *tet D* genes, or genes encoded by ribosomal protection, such as the *tet M*, *tet O*, *tet Q* and *tet S* genes.

**Table.1** PCR amplification conditions for the *tet A* gene

Amplification steps	Conditions temperature/duration <i>tet A</i> Gene
Initial denaturation	95°C / 1 min
Cyclic denaturation	95°C / 30 s
Hybridization	50°C / 30 s
Cyclic elongation	72°C / 20 s
Final elongation	72°C / 7 min
Number of cycles	25

Source: Sunde and Sorum, 2001

**Table.2** Biochemical characteristics determined to confirm the identification of *E. coli* strains

<b>GRAM negative</b>
<b>Mobile peritrich</b>
<b>Pink colonies surrounded by precipitated bile salt halo on MacConkey agar</b>
<b>Positive in indole test</b>
<b>Positive for urea hydrolysis</b>
<b>Negative in citrate test</b>
<b>Negative in oxidase test</b>
<b>Positive for glucose fermentation</b>
<b>Positive for lactose fermentation</b>
<b>Negative hydrogen sulphide</b>
<b>Positive gas production</b>
<b>Positive decarboxylase lysine</b>
<b>Negative desaminase lysine</b>

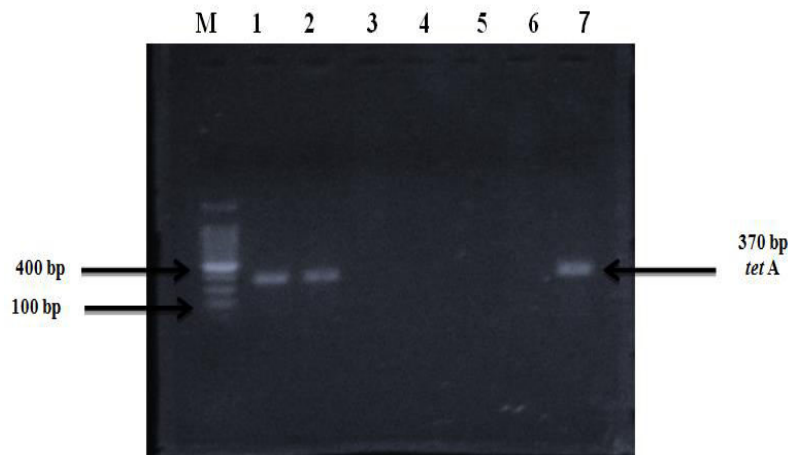
**Table.3** Antibiotic resistance rate of ESBL-producing *E. coli* strains isolated from pig faecal Waste

Antibiotics (concentration µg)	Number of ESBL-producing <i>E. coli</i> isolates (N=35)	Resistance rate (%)
Tetracycline (30)	35	100
Cotrimoxazole (1.25/23.75)	28	80
Gentamicin (10)	25	70
Kanamycin (30)	25	70
Streptomycin (10)	32	90
Ceftriaxone (30)	35	100
Amoxicillin (25)	32	90
Ciprofloxacin (5)	7	20
Nalidixic acid (30)	7	20
Chloramphenicol (30)	3	10
Amoxicillin + clavulanic acid (20/10)	25	70
Colistin (50)	0	0

**Table.4** Distribution of resistance genes detected in ESBL-producing *E. coli* strains

Number of ESBL-producing <i>E. coli</i> (n = 35)	Profile of resistance genes detected
13 (38%)	<i>tet A, bla<sub>TEM</sub></i>
7 (20%)	<i>tet A, bla<sub>TEM</sub>, bla<sub>CTX-M</sub></i>
7 (20%)	<i>tet A, bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>SHV</sub></i>
4 (11%)	<i>tet A, bla<sub>TEM</sub>, bla<sub>SHV</sub></i>
4 (11%)	<i>tet A, bla<sub>CTX-M</sub></i>

**Figure.1** Electrophoretic profile of *tet A* gene amplification product



Line M: Molecular weight marker (Benchtop, 100 bp DNA Ladder, USA);  
 Line 2 and 7: strains analysed harboring *tet A* gene  
 Line 1: Positive control strain

On the other hand, a high prevalence of 97.6% of the *tet A* resistance gene compared with the present study was reported by [Hu et al., \(2013\)](#) in a study carried out in China on *E. coli* isolated from ducks. This high prevalence of the *tet A* resistance gene, amounting to 97.7% in *E. coli* of animal origin, has been reported elsewhere in several countries ([Lanz et al., 2003](#); [Shwaiger et al., 2010](#); [Zhang et al., 2010](#)).

The presence of plasmid-borne *tet A* resistance genes detected in these strains of *E. coli* of animal origin could therefore constitute a health risk for consumers as they would be capable of transferring the gene to commensal bacteria that form part of the human intestinal flora via the food chain or the environment ([Wegener et al., 1999](#)). [Koo and Woo \(2011\)](#) reported in a study that 98.3% of *E. coli* carrying *tet A* resistance genes from animal meat were able to transfer the gene to recipient bacteria.

Another study also reported that multi-resistant bacteria carrying genes encoding resistance to tetracycline, located on plasmids, isolated from wastewater intended for watering plants, transferred their gene horizontally to other bacterial strains ([Sczczepanowski et al., 2004](#)).

The results showed that the strains harboured a specific combination of resistance genes *bla<sub>TEM</sub>, bla<sub>CTX-M</sub>, bla<sub>SHV</sub>, tet A*. The *bla<sub>TEM</sub>, tet A* gene combination was the most predominant with a frequency of 38%.

These cross-resistances would be justified by the fact that the mobile genetic elements contain several antibiotic resistance genes, since the transfer of a single mobile genetic element would consequently lead to the transfer of several antibiotic resistance genes from one bacterium to another through the use of a single antimicrobial agent ([Aarestrup, 2006](#)).

The presence of *tet A* resistance genes in *E. coli* of animal origin such as the pigs in the present study has a negative impact on public health because this gene has the capacity to spread to other commensal bacteria in humans via food or the environment. In addition, the risk of transfer of the *tet A* gene by zoonotic pathogenic bacteria seems very high. Rigorous monitoring of resistance genes circulating in the digestive flora of farmed food-producing animals and good food hygiene practices are therefore necessary to avoid any dissemination of genes from commensal bacteria to pathogenic bacteria.

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### Author Contributions

Kouadio Kouamé Innocent: Conceived the original idea and designed the model and wrote the manuscript and Methodology (microbiological and genotypic analysis); Kouame Amenan Margueritte: Carry out biochemical identification and contribute to the drafting of the manuscript; TRA Bi Youan Charles: Contribute to genotypic study; Ouattara Mohamed Baguy: Visualization; KPODA Stéphane: Data Curation, Validation; Guessennd-Kouadio Nathalie: Project Administration, and Funding Acquisition; AKA Boko: Supervision

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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