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Abstract: The present study aimed at identifying and assessing antimicrobial resistance of Enterococcus spp. isolated from small and medium enterprise slaughterhouses in Kenya. In total, 67 isolates were recovered from 48 of 195 samples examined from beef carcasses, personnel, and cutting equipment in five small and medium enterprise slaughterhouses. The isolates were identified by using matrix-assisted laser desorption-ionization time of flight mass spectrometry and screened thereafter for their resistance against 12 antibiotics by using a disk diffusion assay. The isolates ( n = 67) included Enterococcus faecalis (41.8%), Enterococcus mundtii (17.9%), Enterococcus thailandicus (13.4%), Enterococcus faecium (9.0%), Enterococcus hirae (7.5%), Enterococcus casseliflavus (6.0%), and Enterococcus devriesei (4.5%). None of the isolates were resistant to ciprofloxacin, penicillin, ampicillin, vancomycin, nitrofurantoin, teicoplanin, linezolid, and levofloxacin. Resistance to rifampin (46.3%), erythromycin (23.9%), tetracycline (20.9%), and chloramphenicol (7.5%) was distributed among six of the seven species. All E. thailandicus were resistant to rifampin, erythromycin, and tetracycline. E. faecalis was resistant to rifampin (60.7%), tetracycline (17.9%), erythromycin (14.3%), and chloramphenicol (10.7%). Resistance to two or three antibiotics was observed in 26.9% of the enterococci isolates. The isolation of enterococci that are resistant to clinically relevant antibiotics, such as erythromycin, is of a serious concern given the role enterococci play in the transfer of antibiotic resistance genes.

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Running Head: ***Enterococcus* spp. in Kenyan slaughterhouses**

**Research Note**

# Species distribution and antimicrobial profiles of *Enterococcus* spp. isolates from Kenyan small and medium size enterprises

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Key words: Antimicrobial resistance; *Enterococci*; *E. thailandicus*; Slaughterhouse; Small and medium enterprise

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## ABSTRACT

The present study aimed at identifying and assessing antimicrobial resistance of *Enterococcus* spp. isolated from small and medium enterprise (SME) slaughterhouses in Kenya. In total, 67 isolates were recovered from 48 of 195 samples examined from beef carcasses, personnel and cutting equipment in five SME slaughterhouses. The isolates were identified using MALDI-TOF MS and screened thereafter for their resistance against 12 antibiotics using disk diffusion assay. The isolates (n=67) comprised of *E. faecalis* (41.8%), *E. mundtii* (17.9%), *E. thailandicus* (13.4%), *E. faecium* (9.0%), *E. hirae* (7.5%), *E. casseliflavus* (6.0%), and *E. devriesei* (4.5%). None of the isolates was resistant to ciprofloxacin, penicillin, ampicillin, vancomycin, nitrofurantoin, teicoplanin, linezolid and levofloxacin. Resistance to rifampin (46.3%), erythromycin (23.9%), tetracycline (20.9%), and chloramphenicol (7.5%) was distributed among six of the seven species. All *E. thailandicus* were resistant to rifampin, erythromycin, and tetracycline. *E. faecalis* was resistant to rifampin (60.7%), tetracycline (17.9%), erythromycin (14.3%) and chloramphenicol (10.7%). Resistance to two or three antibiotics was observed in 26.9% of the *Enterococci* isolates. The isolation of *Enterococci* that are resistant to clinically relevant antibiotics, such as erythromycin, is of a serious concern given the role *Enterococci* play in the transfer of antibiotic resistance genes.

*Enterococci* are Gram-positive, catalase-negative, facultative-anaerobic bacteria that form part of the normal intestinal flora and are recognized as one of the leading causes of hospital-associated human infections (*16*). *Enterococci* enter the environment through feces and due to their high adaptability, they easily colonize the soil, water and sewage and subsequently enter raw materials of animal and plant origin (*4*). Their high adaptability also increases their capacity of spreading within the food chain through contaminated foods (*11*). In particular, there is significant potential for their contamination of meat and spread during slaughter since they inhabit the gastrointestinal tract of animals (*9*). In meat and meat products, *E. faecalis* and *E. faecium* have been found to be the most prevalent species (*19*).

The resistance profile of *Enterococci* isolated from animal related sources varies around the globe. For example, *Enterococci* isolated from retail chicken and beef samples in Turkey showed high resistance against tetracycline, erythromycin and ciprofloxacin (*23*). In Canada, *Enterococci* from meat products showed a high prevalence of  clindamycin, tetracycline and tylosin resistance (*9*). Finally, *Enterococci* from Tunisian meat samples showed a high prevalence of tetracycline, erythromycin and streptomycin resistance (*11*). Although not very frequent, emergence of *Enterococci* strains that are resistant to vancomycin, teicoplanin, and linezolid is of particular concern (*16*).

The isolation of antibiotic resistant *Enterococci* from meat, animal related sources and environments associated with animals (*3*), food handling equipment (*7*) and healthy humans (*18*) highlights the need to assess *Enterococci* also in slaughterhouses. *Enterococci* present in slaughterhouses can be transmitted throughout the food chain and colonize intestinal tract of meat consumers.

In Kenya, majority of animals for slaughter are raised in the pastoral areas. In these areas, there have been reported cases of self-medication and misuse of antibiotics, which may contribute to the emergence of antibiotic resistance (*13*). However, there are no documented studies on antibiotic resistant *Enterococci* of foods of animal origin, thus limiting the data available on the prevalence and antibiotic resistance among the *Enterococci* in the country.

The present study aimed at identification of *Enterococcus* spp. isolated along the whole slaughter line as well as from the slaughterhouse environment in small and medium enterprise (SME) slaughterhouses in Kenya and assessing their antimicrobial resistance.

## MATERIALS AND METHODS

**Sampling and *Enterococci* isolation**: *Enterococci* were isolated from 195 swab samples of carcasses, personnel (apron and hands) and cutting equipment (knives and *panga* (African machete)) collected in five SME slaughterhouses in a previous study (*21*). The samples were from the following slaughtering steps: dehiding, evisceration, splitting and dispatch. Appropriate dilutions of the swab samples were spread plated on Chromocult® *Enterococci* agar (Merck, Germany) then incubated for 24 hours at 37 °C.

**Enterococci identification**: From each positive sample, four red colonies that were characteristic for *Enterococci* were selected. These isolates were purified twice on Sheep Blood agar then pre-screened by catalase test. All catalase negative isolates were further identified by MALDI-TOF MS (Bruker BioTyper system version 3.0 (Microflex LT/SH MS)) using α-Cyano-4-hydroxy-cinnamic acid (HCCA) as matrix. The system used FlexiControl and Biotyper real-time classification software (Bruker Daltonics, Bremen, Germany). In cases where two or more of the identified isolates were from the same sample and belonged to the same *Enterococcus* species, only one of these isolates was randomly selected. The selected isolates were stored in 20% glycerol at -20°C for further analysis.

**Antibiotic sensitivity testing**: Antimicrobial resistance was determined using the disc diffusion method according to the Clinical Laboratory Standards Institute (*5*) recommendations. Mueller Hinton agar was used for resistance testing of all species except *E. devriesei*, which was tested using Brain Heart Infusion agar (since there was no growth on Mueller Hinton agar). The isolates were screened for resistance against 12 antibiotics including: ciprofloxacin (CIP: 5 µg), chloramphenicol (CHL: 30 µg), tetracycline (TET: 30 µg), erythromycin (ERY: 15 µg), penicillin (PEN: 10 units), ampicillin (AMP: 10 µg), vancomycin (VAN: 30 µg), nitrofurantoin (NIT: 300 µg), teicoplanin (TEC: 30 µg), rifampin (RIM: 5 µg), linezolid (LZD: 30 µg) and levoflaxacin (LVX: 5 µg).

**Data analysis**: Data was organized in Microsoft Excel 2013 and analysed using SPSS version 23. Descriptive statistics (frequencies, percentages and crosstabs) were used to describe the distribution and antimicrobial resistance profiles of the *Enterococci*.

## RESULTS AND DISCUSSION

**Species distribution**: Sixty-seven isolates were identified as *Enterococci* in 48 out of 195 samples (60 carcasses, 45 aprons, 45 hands, 30 knives and 15 *pangas*) collected in Kenyan SME slaughterhouses. The prevalence on carcass samples, aprons, hands, knives and *pangas* was 23.3%, 28.9%, 26.7%, 16.7% and 26.7%, respectively. Seven *Enterococci* species, which included *E. faecalis* (41.8%), *E. mundtii* (17.9%), *E. thailandicus* (13.4%), *E. faecium* (9.0%), *E. hirae* (7.5%), *E. casseliflavus* (6.0%), and *E. devriesei* (4.5%), were identified. Even though, dominant *Enterococci* vary according to the source of the samples, *E. faecalis* and *E. faecium* mostly dominate in samples associated with animals (*19*). However, the present results showed a higher occurrence of *E. mundtii*. This is in contrast to another study where red meat and fecal samples were analyzed (*11*). *E. mundtii* has so far been rarely isolated from human and environmental samples.

**Antimicrobial resistance rates:** The antimicrobial resistance frequencies and percentage of the identified *Enterococci* isolates are shown in Table 1. Out of the 67 isolates, 56.7% showed resistance against one or more antibiotics. This rate is lower than in another study in Czech Republic that showed a resistance rate of 96% in *Enterococci* isolated from beef carcasses (*17*). Substantial variations in antimicrobial resistance among countries may reflect variation in veterinary antimicrobial usage patterns among the countries (*6*). Although misuse and self-medication have been reported among animal producers in Kenya, the present results may indicate that the use of antibiotics in animal production is not as widespread compared to other countries.

The *Enterococci* isolates showed resistances against four out of the twelve antibiotics. In all the 67 isolates, the rate of resistances against rifampin, erythromycin, tetracycline and chloramphenicol was 43.6%, 23.9%, 20.9% and 7.5%, respectively. In regards to individual species, 100% of *E. thailandicus* were resistant to tetracycline, erythromycin and rifampin while *E. faecalis* were resistant to rifampin (60.7%), tetracycline (17.9%), erythromycin (14.3%) and chloramphenicol (10.7%). Twenty five percent and 50% of *E. casseliflavus* were resistant to erythromycin and rifampin, respectively while 33.3% of *E. faecium* were resistant to rifampin. Finally, 20.0% of *E. hirae* and 7.3% of *E. mundtii* were resistant to both chloramphenicol and erythromycin. *Enterococci* are highly adaptable and have the ability to develop resistances against most antimicrobial used against them in response to selective pressure. For this reason, the introduction and widespread use of chloramphenicol, erythromycin and tetracycline, corresponded with the emergence of *Enterococci* resistant against these antibiotics (*12*). This may indicate that these antibiotics or other antibiotics that are within the same group are commonly used in Kenya.

According to reports, resistance of *Enterococci* against rifampin and erythromycin is quite common especially in samples associated with animals (*19*). Resistance observed in the present study may be attributed to the use of some of these antibiotics in animal production and may reflect their use in the country. In particular, tetracycline is one of the most widely used antibiotic in food producing animals in Kenya (*15*). In the present study, resistance against rifampin was higher than resistance against antibiotics commonly used in livestock production. Rifampin is banned in livestock production hence there is no direct selective pressure. However, it was previously reported that rifampin resistance can occur as a result of spontaneous mutations or from co-selection in the presence of floroquinolones commonly used in livestock production (*14*).

All isolates were described as either intermediate resistant or susceptible to ciprofloxacin, penicillin, ampicillin, vancomycin, nitrofurantoin, teicoplanin, linezolid and levofloxacin. Most of these antibiotics are used to treat human Enterococcal infections. For example, ampicillin is the most commonly used antibiotic and can also be used to treat complicated urinary tract infections (*20*). On the other hand, linezolid is used to treat infections caused by *E. faecium* that are resistant to vancomycin (*2*).

The distribution of antibiotic resistant *Enterococci* in the samples and slaughter stages in the SME slaughterhouses is shown in Table 2. The five (7.5%) isolates that were resistant to chloramphenicol were isolated in personnel hands in two SME slaughterhouses (S2 and S4). One isolate was from the evisceration stage, while the other four were equally distributed between flaying and splitting stages. The 14 (20.9%) and 16 (23.9%) isolates that were resistant against tetracycline and erythromycin were from carcasses, hands and aprons. At least one of these resistant isolates was from each of the four slaughter stages. While isolates resistant against tetracycline were isolated in all slaughterhouses, isolates resistant against erythromycin were isolated in all slaughterhouses except one (S5). The 31 (43.6%) isolates that were resistant against rifampin were isolated from carcasses, hands, apron, knives and panga distributed in all the slaughterhouses and slaughter stages. A previous study reported that resistant *Enterococci* were present in samples collected after carcass evisceration and during meat processing (*17*). The present study showed that resistant *Enterococci* are also distributed among the various samples within the slaughterhouse and slaughter process stages.

**Antimicrobial resistance profiles**: The antimicrobial resistance patterns of *Enterococci* isolated from the Kenyan SME slaughterhouses are shown in Table 3. About 30% of the isolates were resistant against only one antibiotic. On the other hand, resistance against two or three antibiotics was observed in 26.9% of the *Enterococci*. The majority (14.9%) of the isolates that had multiple resistance were resistant against three antibiotics compared to 11.9% of the isolates that were resistant against two antibiotics. These results correspond with a previous report that the rate of multiple antibiotic resistance in *Enterococci* is low especially in environmental samples compared to clinical samples (*1*). The rate of multi resistance in the present study was, however, lower than in another study where the rate was observed in more than half of the isolates (*8*).

Two isolates were resistant against chloramphenicol, erythromycin and erythromycin. Multiple resistance against the pairs of antibiotics namely chloramphenicol and rifampin, chloramphenicol and tetracycline, erythromycin and rifampin, and tetracycline and erythromycin was observed in one isolate for each pair. Multiple resistance against chloramphenicol, tetracycline and erythromycin was also observed in one isolate. Multiple resistance in *Enterococci* isolated from meat has been reported against five antibiotics (*10*) compared to the present study in which the highest number of resistances was three. Multiple resistance against three antibiotics tetracycline, erythromycin and rifampin was observed in nine *E. thailandicus* isolates. A recent genome announcement also reported multiple resistance genes in *E. thailandicus* isolated from sewage (*22*). This is the first time that multiple resistance in *E. thailandicus* is being reported in isolates associated with food processing facilities.

This study is the first report on the distribution and antimicrobial resistance of *Enterococci* isolated from carcasses, personnel and equipment at different slaughter stages in Kenyan SME beef slaughterhouses. The *Enterococci* belonged mainly to the species *E. faecalis*, *E. mundtii* and *E. thailandicus*. The isolates showed no resistances against antibiotics commonly used to treat human Enterococcal infections such as vancomycin, penicillins and linezolid. The isolates were, however, resistant against erythromycin, tetracycline, chloramphenicol and rifampin. With the exception of tetracycline, these antibiotics are used to treat human infections. Antibiotic resistant *Enterococci* present in slaughterhouses can be transmitted throughout the food chain and colonize the intestinal tract of meat consumers.

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TABLE 1. Antimicrobial resistance frequencies (percentages) of *Enterococcus* spp. (n=67) isolated in Kenyan small and medium enterprises

| Antibiotics | *E. casseliflavus** | *E. devriesei* | *E. faecalis* | *E. faecium* | *E. hirae* | *E. mundtii* | *E. thailandicus* | Total |
|---|---|---|---|---|---|---|---|---|
| Ciprofloxacin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Chloramphenicol | 0 (0.0) | 0 (0.0) | 3 (10.7) | 0 (0.0) | 1 (20.0) | 1 (7.3) | 0 (0.0) | 5 (7.5) |
| Tetracycline | 0 (0.0) | 0 (0.0) | 5 (17.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 9 (100.0) | 14 (20.9) |
| Erythromycin | 1 (25.0) | 0 (0.0) | 4 (14.3) | 0 (0.0) | 1 (20.0) | 1 (7.3) | 9 (100.0) | 16 (23.9) |
| Penicillin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Ampicillin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Vancomycin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Nitrofurantoin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Teicoplanin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Rifampin | 2 (50.0) | 0 (0.0) | 17 (60.7) | 2 (33.3) | 1 (20.0) | 0 (0.0) | 9 (100.0) | 31 (46.3) |
| Linezolid | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Levoflaxacin | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

*Figures in brackets are percentages of resistant isolates out of the total number of isolates per species

TABLE 2. Distribution of antibiotic resistant *Enterococcus* spp. in Kenyan small and medium scale slaughterhouses.

| AB | Samples | Slaughterhouses* S1 | S2 | S3 | S4 | S5 | Slaughter stages |
|---|---|---|---|---|---|---|---|
| CHL | Hands | – | 2 | – | 3 | – | Flaying and splitting |
| TET | Carcasses | 1 | 1 | – | 3 | – | Flaying, evisceration, splitting and dispatch |
| | Clothes | 2 | – | 1 | – | – | |
| | Hands | 2 | 1 | 1 | 1 | 1 | |
| ERY | Carcasses | 1 | 1 | – | 2 | – | Flaying, evisceration, splitting and dispatch |
| | Clothes | 2 | – | 1 | – | – | |
| | Hands | 2 | 2 | 1 | 4 | – | |
| RIF | Carcasses | 1 | 1 | – | 3 | 1 | Flaying, evisceration, splitting and dispatch |
| | Clothes | 2 | – | 1 | 2 | 2 | |
| | Hands | 3 | 1 | 1 | 6 | 1 | |
| | Knives | – | – | – | 1 | 2 | |
| | Panga | – | 2 | – | 1 | – | |

AB: Antibiotics; CHL: Chloramphenicol; TET: Tetracycline; ERY: Erythromycin; RIF: Rifampin
*– no resistant *Enterococcus* spp. were identified in the specific slaughterhouse samples

TABLE 3. Antimicrobial resistance profiles of *Enterococcus* spp. (n=67) isolated in Kenyan small and medium slaughterhouses

| Number of resistances | *E. casseliflavus* | *E. devriesei* | *E. faecalis* | *E. faecium* | *E. hirae* | *E. mundtii* | *E. thailandicus* | Resistance phenotype |
|---|---|---|---|---|---|---|---|---|
| 0 | 2 | 3 | 6 | 4 | 3 | 11 | – | - |
| 1 | – | – | 1 | – | – | – | – | ERY |
| | 1 | – | 12 | 2 | 1 | – | – | RIF |
| | – | – | 1 | – | – | – | – | TET |
| 2 | – | – | 1 | – | – | 1 | – | CHL–ERY |
| | – | – | 1 | – | – | – | – | CHL–RIF |
| | – | – | – | – | 1 | – | – | CHL–TET |
| | 1 | – | – | – | – | – | – | ERY–RIF |
| | – | – | 1 | – | – | – | – | TET–ERY |
| | – | – | 2 | – | – | – | – | TET–RIF |
| 3 | – | – | 1 | – | – | – | – | CHL–TET–ERY |
| | – | – | – | – | – | – | 9 | TET–ERY–RIF |

CHL: Chloramphenicol; TET: Tetracycline; ERY: Erythromycin; RIF: Rifampin
– No resistant *Enterococcus* spp. were identified