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# Vancomycin-Resistant Enterococcus spp. (VRE) from Chicken cloacal Swab in Gaza Strip Poultry Farms

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**Abstract**

Vancomycin Resistant Enterococci (VRE) is increasingly becoming a major problem during the last years, especially in health care settings. Poultry is known for being a source of many pathogens that cause infections to human and concerns of transmitting VRE are justified. The main objective of this study is to determine the presence of Vancomycin-Resistant Enterococcus Spp. from chicken cloacal swab in Gaza strip farms, Palestine. Cloacal samples were collected from live broiler chicken from different locations and cultured onto Bile Esculin Agar plates. All isolates were identified and confirmed by conventional microbiological tests and biochemical characterization was performed using API20 Strep. Disk diffusion method was used to determine the antimicrobial sensitivity of isolates against six antimicrobial agents. Enterococcus mund was the most frequently isolated Enterococcus species (31%). Wide variations were observed in the susceptibilities of the different isolates to antimicrobials. 92% of the isolates were resistance to Tetracycline, followed by Penicillin (79%), Ampicillin (71%), Levofloxacin (61%), Fosfomycin (57%), and Vancomycin (44%). Results of the present study showed high resistance rates among Enterococci isolates thus the role of chicken as a source of resistant bacteria that may get into human food chain, leading to human infection should be investigated.

**Keywords:**

*Antimicrobial resistance, Chicken Cloacal swab, VRE, Gaza strip, Palestine.*

## 1. Introduction:

*Enterococcus* spp. are resistant to a wide variety of antibiotics. This feature allows enterococci to survive in hospital environment, where antibiotics are used, and provides the opportunity for dissemination of resistant organisms (Murray 1990). This resistance can be both intrinsic, mediated by genes located on the chromosome – a characteristic present in almost all the strains of enterococci-, or acquired, mediated by genes residing on plasmids or transposons (Barbosa, Ferreira et al. 2009).

Resistance to cephalosporins, sulphonamides, lincosamides, many ß-lactams and low levels of aminoglycosides, is recognized as an intrinsic resistance (Murray 1990). The acquired genetic determinants confer resistance to all classes of antimicrobials, including chloramphenicol, tetracyclines, erythromycin, rifampicin, ampicillin and glycopeptides. The main concern is that the

genes coding for all these antibiotic resistance traits could be transferred by pheromone mediated, conjugative plasmids or transposons to other enterococci or even to more virulent pathogens (Schwarz, Perreten et al. 2001).

Bacteria of the genus *Enterococcus* are important human pathogens that are frequently resistant to a number of clinically important antibiotics. They are also used as markers of animal fecal contamination of human foods and are employed as sentinel organisms for tracking trends in resistance to antimicrobials with Gram-positive activity (Tyson, Nyirabahizi et al. 2018).

VanA glycopeptide resistance is characterized by acquired inducible resistance to both vancomycin and teicoplanin, whereas the VanB phenotype is characterized by variable levels of resistance to vancomycin but susceptibility to teicoplanin. Vancomycin-resistant *Enterococcus faecium* (VRE-fm) strain with VanB phenotype-*vanA* genotype incongruence was reported (Park, Lee et al. 2008). The occurrence of VRE-fm in food is relevant to public health, as foodborne it may colonize the gut of consumers and transfer vancomycin resistance genes to the indigenous gut microbiota (Leinweber, Alotaibi et al. 2018).

Enterococci, including *E. faecalis* and *E. faecium*, are present in the guts of food-producing animals and are used as a measure of fecal contamination of meat (Tyson, Nyirabahizi et al. 2018). Enterococci are commensal bacteria in the intestines of humans and domestic animals, but they can also be detected in the environment, from soil, water, plants, wild animals, birds, and insects. In humans, *E. faecalis* and *E. faecium* can cause urinary tract infections, wound infections, bacteraemia, and infective endocarditis. Resistant enterococci are selected both in humans and in animals, owing to the use of antimicrobial agents in both settings (Hammerum 2012) .

Newer antibiotics such as linezolid, daptomycin and tigecycline have good *in vitro* activity against enterococcal isolates, although their clinical use may be limited in certain clinical scenarios as a result of reduced rates of success, possible underdosing for enterococci, and low serum levels, respectively, and also by the emergence of resistance (Arias, Contreras et al. 2010)

The aim of this study was to isolate, characterize and investigate the resistance of *Enterococcus* isolates from poultry samples to various antimicrobial agents including the glycopeptide (vancomycin).

## 1. Materials and Methods

### Media, Reagents

Both commercially available general and selective media were used for the storage, growth and characterization of the *Enterococcus* species (Muller Hinton Agar, Blood agar, Brain heart infusion, Triple sugar iron agar, and Bile Esculin Agar). The media were prepared in the laboratory as per direction of the manufacturers.

### Sample sources

Samples were collected from three areas of Gaza strip (Middle governorates, Gaza, and South Gaza (Rafah and Khan Younes). Only one sample type was collected (chicken rectal swab).

### Culture in Bile Esculin Agar

Chicken cloacal swab was streaked onto the surface of Bile Esculin Agar and Blood agar plates and incubated at 37°C for 24h. Suspected colonies of *Enterococcus* were observed as slightly gray, small and partially blackening the media. Suspect colonies were subcultured onto Blood agar (BA) media for

pure culture isolation and biochemical characterization.

**Pure culture storage**

Isolated bacteria were cultured in BHI broth at 37°C. After 24h incubation period of growth, cultures were stored at -80°C after preparing 25% glycerol stock in a 1.5 ml vials for long-term preservation and TSIA slants were used for the routine maintenance of the pure bacterial strains as short-term preservation at -20°C.

**Biochemical tests**

Identification of the strains was further investigated to the genus level by biochemical reactions (Catalase test and Esculin hydrolysis) as described by MWOVA (MWOVA 2016).

**Identification**

**APi-20 Strep inoculation and interpretation**

Gram positive cocci that were shown to be catalase negative were further identified. The isolates were phenotypically identified using API20 STREP (bio Meraux, France). A pure growth of the test organism was suspended in 2 ml of the API GP Medium. The strep was inoculated as recommended by the manufacturer. The results of the API20 STREP were interpreted according to the chart provided.

**Antimicrobial susceptibility assay**

The susceptibility of isolates to various antimicrobials was determined using the disk diffusion method recommend by Clinical laboratory Sciences Institute (CLSI, 2017). McFarland 0.5 turbidity standard was prepared as per the standard guidelines described by the (Cockerill, Clinical et al. 2010). At least 3-5 well isolated colonies of the same morphological type were selected from the blood agar plate culture. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4 ml normal saline. The broth tube was adjusted to the McFarland standards. A sterile cotton swab was dipped into the adjusted suspension. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the swab over the entire agar surface. Antimicrobial discs (Table 1) were dispensed onto the surface of the inoculated agar plate. The plates were incubated for 24 hours at 37 °C and the zone of inhibition of each antimicrobial was measured and recorded. Interpretation as sensitive, resistant and intermediate are done according the interpretation chart provided by the manufacturer.

**Table (1)** Antimicrobials tests against Enterococcal isolates from poultry samples.

| Antimicrobial | Abbreviation | Concentration |
|---|---|---|
| **Ampicillin** | AMP | 10 µg |
| **Fosfomycin** | FF | 200 µg |
| **Levofloxacin** | LEV | 5 µg |
| **Penicillin** | P | 10 µg |
| **Tetracycline** | TE | 30 µg |
| **Vancomycin** | VA | 30 µg |

## Data analysis

Collected data were tabulated and analyzed using Statistical Package for Social Sciences (SPSS v.22) software. The results are presented through histograms, tables and pie charts.

## 2. Results

### Bacterial isolates and identification results

All samples, 100 (100%) yielded positive growth on Bile Esculin agar and Blood agar media. A total of 100 Gram-positive bacteria isolates were examined for catalase enzyme. All were catalase negative. All the tested 100 samples showed esculin hydrolysis by blackening the media (Figure 1).

**Figure (1)** Growth characteristic of isolated *Enterococcus* spp. On bile esculin agar media.

### Biochemical characterizations

The results of the biochemical identification showed that the predominant bacterial isolate was *E. mund* (31%), followed by *E. gallin* (26%), *E. malod* (22%), followed by *E. cassel* (6%), *E. faecalis* (5%), *E. columbae* (4%), *E. asini* (2%) *and E. flaves* (1%) (Table 2).

**Table (2)** *Enterococcus spp.* isolated from cloacal swabs.

| Identification | Frequency |
|---|---|
| ***E. mund*** | 31 |
| ***E. gallin*** | 26 |
| ***E. malod*** | 22 |
| ***E. cassel*** | 6 |
| ***E. faecalis*** | 5 |
| ***E. columbae*** | 4 |
| ***E. faecium*** | 3 |
| ***E. asini*** | 2 |
| ***E. flaves*** | 1 |
| **Total** | 100 |

### Antimicrobial susceptibility assay result

In this study, 100 Enterococcal isolates were tested against six commercially available antibiotic disk (table 1). The sizes of zones of inhibition of every antibiotic disc were measured in millimeter and compared with zone diameter interpretive standards from CLSI 2018 for *Enterococcus.* Isolates were classified as sensitive, intermediate or resistant (Table 3). Among the tested isolates, none was sensitive to all antibiotics whereas 100% showed resistance to at least three antibiotics.

The majority of the *Enterococcus* spp. isolates showed variations in their susceptibility to different antimicrobials. Resistance to vancomycin is 44%. High rates of resistance were observed against Tetracycline (92%), followed by penicillin (79%), and ampicillin (71%) were also recorded (Table 3).

**Table (3)** Percentage of *Enterococcus* spp. isolates resistance to the tested antimicrobial agent

| Antimicrobial | % | | |
|---|---|---|---|
| | R | S | I |
| **Ampicillin** | 71 | 29 | 0 |
| **Fosfomycin** | 57 | 40 | 3 |
| **Levofloxacin** | 61 | 13 | 26 |
| **Penicillin** | 79 | 21 | 0 |
| **Tetracycline** | 92 | 5 | 3 |
| **Vancomycin** | 44 | 52 | 4 |

*E. faecium* and *E. asini* showed the highest resistance to vancomycin (100%) followed by *E. cassel* (83.3%) and the least resistant was *E. flaves*. In general, the human pathogenic species *E. faecium* was completely resistant to 4 of the antibiotics and was completely susceptible to Fosfomycin. The other human pathogen, *E. faecalis*, was also highly resistant to three antibiotics (80%) and moderately resistant to the other three (40%). Tetracycline resistance was found to be a common theme among all isolates (Table 4).

**Table (4)** Susceptibility rates of enterococci isolated from chickens

| Bacteria | % of Resistance | | | | | | |
|---|---|---|---|---|---|---|---|
| | N | VA | P | TE | AMP | LEV | FF |
| ***E. malod*** | **2** | 8 | 17 | 21 | 15 | 12 | 14 |
| | **2** | 36.4 % | 77.3 % | 95.5 % | 68.2 % | 54.5 % | 63.6 % |
| ***E. gallin*** | **2** | 9 | 21 | 23 | 19 | 17 | 17 |
| | **6** | 34.6 % | 80.8 % | 88.5 % | 73.1 % | 65.4 % | 65.4 % |
| ***E. cassel*** | **6** | 5 | 5 | 6 | 5 | 6 | 2 |
| | | 83.3 % | 83.3 % | 100 % | 83.3 % | 100 % | 33.3 % |
| ***E.mund*** | **3** | 12 | 23 | 28 | 19 | 19 | 19 |
| | **1** | 38.7 % | 74.2 % | 90.3 % | 61.3 % | 61.3 % | 61.3 % |
| ***E. faecium*** | **3** | 3 | 3 | 3 | 3 | 2 | 0 |
| | | 100 % | 100 % | 100 % | 100 % | 66.7 % | 0.0% |
| ***E. columbae*** | **4** | 3 | 3 | 4 | 4 | 3 | 2 |
| | | 75% | 75% | 100 | 100 | 75% | 50% |
| ***E. faecalis*** | **5** | 2 | 4 | 4 | 4 | 2 | 2 |
| | | 40% | 80% | 80% | 80% | 40% | 40% |
| ***E. flaves*** | **1** | 0 | 1 | 1 | 0 | 0 | 1 |
| | | 0.0% | 100 % | 100 % | 0.0% | 0.0% | 100 % |
| ***E. asini*** | **2** | 2 | 2 | 2 | 2 | 0 | 0 |
| | | 100 % | 100 % | 100 % | 100 % | 0.0% | 0.0% |

The resistance profile of MDR isolates ranged from 3 to 6 antibiotics. The highest percentages for MDR isolates were from *E. mund* (30.9%), followed by *E. gallin* (25.7%), and *E. malod* (21.6%). Among the 100 isolates 97% were MDR.

**Table (5):** Multiple antibiotic resistance of *Enterococuss* spp.

| *Isolate* | MDR | | Non-MDR | |
|---|---|---|---|---|
| | N | % | N | % |
| ***E. malod*** | 21 | 95 | 1 | 5 |
| ***E. gallin*** | 25 | 96 | 1 | 4 |
| ***E. cassel*** | 6 | 100 | 0 | 0 |
| ***E. mund*** | 30 | 97 | 1 | 3 |
| ***E. faecium*** | 3 | 100 | 0 | 0 |
| ***E. columbae*** | 4 | 100 | 0 | 0 |
| ***E. faecalis*** | 5 | 100 | 0 | 0 |
| ***E. flaves*** | 1 | 100 | 0 | 0 |
| ***E. asini*** | 2 | 100 | 0 | 0 |
| ***Total*** | 97 | 97 | 3 | 3 |

## 3. Discussion

*Enterococcus* spp. are widely distributed in nature and are associated with the spoilage of meat and meat products (Hugas, Garriga et al. 2003). Worldwide prevalence and outbreaks of *Enterococcus* species are increasing day by day. Reports of several Enterococci outbreaks in broiler farms especially in Europe and Americas increases its significance throughout the world (Cockerill, Clinical et al. 2010). In addition, enterococci can rapidly acquire antimicrobial resistance through mutations or acquisition of plasmids and

transposons that contain foreign genetic material, including vancomycin-resistance genes. Outbreaks of vancomycin-resistant enterococci (VRE) in humans may be associated with VRE in livestock, although the transmission mechanisms remain unclear (da Costa, Loureiro et al. 2013). Enterococci play both as commensals and as pathogens because of its many virulence factors (VFs) associated with capsule formation including gelatinase, enterococcal surface protein, biofilm formation, and aggregation substance (Hammad, Shimamoto et al. 2014).

The main objectives of current study were to isolate and characterize *Enterococcus* spp. from commercial broiler chickens in Gaza and to check their antimicrobial resistance profiles against currently available antibiotics in Gaza. Two studies in Gaza strip investigated the Vancomycin-resistant against Enterococci isolated from human fecal materials (Elmanama 2008; Hijazi, Elmanama et al. 2009) .

In this study, the predominant bacterial isolate was *E. mund* (31%), and the least was *E. flaves* (1%) (Table 2). The existence of such isolates in the food chain of humans is of a great concern not only to public health but also because of the ease of resistance gene transfer to other bacteria. The result obtained by Jørgensen and others indicate a higher *E. faecalis* rates among cloacal swabs isolates. The prevalence of *E. faecalis* from two confined flocks was 83 and 96%, while only 44 and 13% in two fancy breeder flocks (Jørgensen, Poulsen et al. 2017). Another study, showed that the most commonly identified species were *Enterococcus* (*E. faecalis*) (74.7%), *E. faecium* (10.1%), *E. gallinarum* (5.5%), *E. hirae* (4.6%), and *E. cecorum* (4.1%) (Stępień-Pyśniak, Marek et al. 2016).

Chingwaru et al., studied 1,467 *Enterococci* isolated from the milk, beef, and chicken samples, they found *E. faecalis* (46.1%) and *E. faecium* (29.0%) to be the predominant species (Chingwaru, Mpuchane et al. 2003). Yielding a total of 21,077 *Enterococcus* isolates. *E. faecalis* and *E. faecium* constituted the majority of the *Enterococcus* isolated from retail meat (64.0% and 28.6%, respectively) (Tyson, Nyirabahizi et al. 2018).

In our study, *Enterococcus* isolates were tested for resistance to six antimicrobials, some of whci are used in both human and veterinary medicine to treat infections caused by *Enterococcus* and other gram-positive organisms. Vancomycin has long been considered an important treatment for Enterococcus infections. Although vancomycin resistance among *E. faecium* in United States hospitals can top 80% (63). In this study, resistance of *E. faecium* to vancomycin is 100%. This is an interesting finding because Vancomycin and other glycopeptides are not supposed to be used in poultry. The acquisition and spread of vancomycin resistance in poultry needs to be investigated.

Resistance to vancomycin is widespread. It was found that 13.1% of the *E. faecalis* isolates from chicken samples. In contrast, 30.7% of the *E. faecium* isolates from chicken samples, were resistant to vancomycin (Chingwaru, Mpuchane et al. 2003). No significant resistance was detected for vancomycin in an Italian study (Bertelloni, Salvadori et al. 2015). In a study from Libya, only two isolates out of six (33.3%) were resistant to gentamycin and vancomycin (Naas, Almajdoubi et al. 2017). Only 33.3% of the isolates resistant to vancomycin and gentamicin were recorded in a Canadian study (Jahan, Krause et al. 2013)..

The spread of opportunistic pathogens harboring vancomycin resistance genes into community is a potential threat to public health as vancomycin is used as the last-resort against many infections.

In our study the high rates of resistance were observed against Tetracycline (92%). Tetracycline is not typically used to treat *Enterococcus* infections, although tetracyclines represent the drug class with the highest sales for use in food-producing animals (FDA 2016). In Brazil, a study reported an overall 31.2% resistance to tetracycline (Fracalanzza,

Scheidegger et al. 2007), which is very much lower than reported in this study.

In our study, most (97%) of the isolated *Enterococci* spp. strains were MDR. Researchers investigating *Enterococci* isolated from captive baboons in Nairobi found resistance to multiple drugs in relatively large numbers (MWOVA 2016). Lower rates of MDR were reported by an Italian study who classified 53% of their isolates as multidrug-resistant (MDR) and 6 (5.2%) strains as possibly extensively drug-resistant (XDR). *E. faecium* was the most prevalent antimicrobial resistant species, followed by *E. faecalis* and *E. durans* (Bertelloni, Salvadori et al. 2015). MDR pattern of the current study was quite similar with the other studies (Sørensen, Blom et al. 2001; Khan, Nawaz et al. 2005; Dolka, Chrobak-Chmiel et al. 2016) where high level of MDR isolates were observed too. Variation in resistance among species of the same genus do exist. Genetic makeup, exposure to antimicrobials and other environmental factors may explain such variations.

## Conclusions

The results demonstrated that the risk of dissemination of multi-drug resistant enterococci from chicken is real, especially VRE. Thus, farms, that have common outside environment with people, could represent a hazard for public health. There is a need to implement measures which guarantee or reduce misuse of antimicrobial drugs in chicken feeds and treatment in order to minimize the emergence and dissemination of antibiotic resistant clones to humans in close contact with chickens and chicken farms. It is also essential to educate farmers about the antibiotic applications in animal farms including poultry.

## References

Arias, C. A., G. A. Contreras, et al. (2010). "Management of multidrug-resistant enterococcal infections." Clinical Microbiology and Infection **16**(6): 555-562.

Barbosa, J., V. Ferreira, et al. (2009). "Antibiotic susceptibility of enterococci isolated from traditional fermented meat products." Food Microbiology **26**(5): 527-532.

Bertelloni, F., C. Salvadori, et al. (2015). "Antimicrobial resistance in Enterococcus spp. isolated from laying hens of backyard poultry flocks." Annals of Agricultural and Environmental Medicine **22**(4).

Chingwaru, W., S. Mpuchane, et al. (2003). "Enterococcus faecalis and Enterococcus faecium isolates from milk, beef, and chicken and their antibiotic resistance." Journal of food protection **66**(6): 931-936.

Cockerill, F. R., Clinical, et al. (2010). Performance standards for antimicrobial susceptibility testing: twentieth informational supplement;[... provides updated tables for... M02-A10 and M07-A8], National Committee for Clinical Laboratory Standards.

da Costa, P. M., L. Loureiro, et al. (2013). "Transfer of multidrug-resistant bacteria between intermingled ecological niches: the interface between humans, animals and the environment." International journal of environmental research and public health **10**(1): 278-294.

Dolka, B., D. Chrobak-Chmiel, et al. (2016). "Phenotypic and genotypic characterization of Enterococcus cecorum strains associated with infections in poultry." BMC veterinary research **12**(1): 129.

Elmanama, A. A. (2008). "Vancomycin Resistant Enterococci (VRE) among Non–Hospitalized Individuals in Gaza City, Palestine." Age **1**(16): 17-40.

FDA, U. (2016). "2015 summary report of antimicrobial sold or distributed for use in food-producing animals."

Hammad, A. M., T. Shimamoto, et al. (2014). "Genetic characterization of antibiotic resistance and virulence factors in Enterococcus spp. from Japanese retail ready-to-eat raw fish." Food microbiology **38**: 62-66.

Hammerum, A. (2012). "Enterococci of animal origin and their significance for public health." Clinical Microbiology and Infection **18**(7): 619-625.

Hijazi, N., A. A. Elmanama, et al. (2009). "Vancomycin-resistant enterococci in fecal samples from hospitalized patients and non-hospitalized individuals in Gaza City." Journal of Public Health **17**(4): 243-249.

Hugas, M., M. Garriga, et al. (2003). "Functionalty of enterococci in meat products." International journal of food microbiology **88**(2-3): 223-233.

Jahan, M., D. O. Krause, et al. (2013). "Antimicrobial resistance of Enterococcus species from meat and fermented meat products isolated by a PCR-based rapid screening method." International journal of food microbiology **163**(2-3): 89-95.

Jørgensen, S., L. Poulsen, et al. (2017). "Characterization of Enterococcus faecalis isolated from the cloaca of 'fancy breeds' and confined chickens." Journal of applied microbiology **122**(5): 1149-1158.

Khan, S. A., M. S. Nawaz, et al. (2005). "Molecular characterization of multidrug-resistant Enterococcus spp. from poultry and dairy farms: detection of virulence and vancomycin resistance gene markers by PCR." Molecular and cellular probes **19**(1): 27-34.

Leinweber, H., S. M. Alotaibi, et al. (2018). "Vancomycin resistance in Enterococcus faecium isolated from Danish chicken meat is located on a pVEF4-like plasmid persisting in poultry for 18 years." International journal of antimicrobial agents.

Murray, B. E. (1990). "The life and times of the Enterococcus." Clinical microbiology reviews **3**(1): 46-65.

MWOVA, J. P. (2016). ANTIMICROBIAL RESISTANCE GENES HARBOURED IN ENTEROCOCCI ISOLATED FROM THE FAECES OF CAPTIVE BABOONS, UNIVERSITY OF NAIROBI.

Naas, H. T., Z. Almajdoubi, et al. (2017). "MOLECULAR IDENTIFICATION AND ANTIBIOGRAM OF Enterococcus spp. ISOLATED ON ENTEROCOCCUS SELECTIVE DIFFERENTIAL (ESD) MEDIA FROM MEAT, MEAT PRODUCTS AND SEAFOOD IN LIBYA." The Journal of Microbiology, Biotechnology and Food Sciences **6**(6): 1264.

Park, I. J., W. G. Lee, et al. (2008). "VanB phenotype-vanA genotype Enterococcus faecium with heterogeneous expression of teicoplanin resistance." Journal of clinical microbiology **46**(9): 3091-3093.

Schwarz, F. V., V. Perreten, et al. (2001). "Sequence of the 50-kb conjugative multiresistance plasmid pRE25 from Enterococcus faecalis RE25." Plasmid **46**(3): 170-187.

Sørensen, T. L., M. Blom, et al. (2001). "Transient intestinal carriage after ingestion of antibiotic-resistant Enterococcus faecium from chicken and pork." New England Journal of Medicine **345**(16): 1161-1166.

Stępień-Pyśniak, D., A. Marek, et al. (2016). "Prevalence and antibiotic resistance of Enterococcus strains isolated from poultry." Acta Veterinaria Hungarica **64**(2): 148-163.

Tyson, G. H., E. Nyirabahizi, et al. (2018). "Prevalence and antimicrobial resistance of enterococci isolated from retail meats in the United States, 2002 to 2014." Applied and environmental microbiology **84**(1): e01902-01917.