

Comparison between different methods diagnostic *Enterococcus* species

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Summary

One hundred and five isolates of *Enterococcus* species were identification from 705 samples of urine collected from UTI patient in Baghdad from period between February - may 2014. Azide blood agar and esculin media were used for preliminary identification of *Enterococcus* while chromogenic agar orientation medium as used as confirmatory media for this paper modification media showed that 55 isolates of *Enterococcus* were *Enterococcus faecalis* and 50 isolates were *Enterococcus faecium*. PCR technique using *ddlE.faecalis* and *ddl E.faecium* proved that all isolates of *E.faecalis* and *E.faecium* previously identification successfully procedure PCR amplification product i.e. conform the previously diagnosis.

Introduction

Enterococci are causing UTI and consider as a second causative agent following *Staphylococcus aureus* of endocarditis and bacterimia(1). They are normal inhabitants of the gastrointestinal tract of humans and animals. Medical importance species of a *Enterococcus*, *Enterococcus faecalis* and *E. faecium*. In 1984 the enterococcus faecalis was named *streptococcus faecalis* then reorganized into *Enterococcus faecalis*. The relative importance of *E. faecium* as a pathogen has increased with the occurrence of high-level resistance to multiple antimicrobial drugs, such as ampicillin and vancomycin (2).

Vancomycin-resistant *Enterococcus*, (VRE), are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic vancomycin, The first VRE isolates that harbored the *vanA* transposon were identified in 1987 in Europe (3,4), and within 10 years VRE represented more than 25% of Enterococci associated with bloodstream infections in hospitalized patients in the United States (1).

Reported prevalence of VRE in hospitals was been low, but increasing rates (>10%) in stool and clinical samples were reported recently (5–6).

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However, nosocomial VRE infection and transmission have occurred much more frequently in the United States. Recent reports have documented, in hospitalized patients, horizontal transfer of the *vanA* gene from vancomycin-resistant *E. faecalis* to methicillin-resistant *Staphylococcus aureus* (MRSA), creating MRSA with high-level resistance to vancomycin (7–8).

This study aimed to make comprised between classical culture media and chromogenic agar with PCR technique in identification of *Enterococcus* species.

Material & methods

Collection of sample

A total Of 705 urine sample were collected from different Hospitals in Baghdad during a period between February - may 2014 .the samples were obtained from mid stream urine from patient suffering from urinary tract infections.

Culture media

azide blood agar and bile esculin media. This media were used for preliminary identification of members of genus *Enterococcus* This media were praper according to manufacturer's instruction .

Confermative identifecation was done a following:

1-culture media

A-CHROMO agar orientation

This medium praper according to manufacturer's instruction ,membares of genus *Enterococcus* appear as trquze color.

B-Modified Cephalixin-Aztreonam-Arabinose Agar_:

Standard cephalixin-aztreonam arabinose agar (CAA), which consisted of 40g of Columbia agar base, 10g of arabinose, 3.6ml of phenol red (2%), 75mg/liter of aztreonam and 50mg/liter of cephalixin [Ford et al 1994] was substituted by modified cephalixin-aztreonam arabinose agar as in table (1), which was prepared by (9), and used as a modification for the above medium by using azide blood agar base 33.2g added to it 10g of arabinose and 2% phenol red solution (3.6ml/L). In this modified medium, antibiotic aztreonam was removed and substituted by 0.20g of sodium azide to give no chance for all gram negative bacteria to grow on this medium. The medium was mixed, and the pH was adjusted to 7.8 . The agar was autoclaved at 121°C and pressure 15 psi for 18 min. Fresh sterile solution of antibiotic cephalixin was added to the medium to a final concentration of 50 mg/L. The medium was poured into sterile petri dishes.

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Table (1): Modified Cephalixin-Aztreonam-Arabinose agar medium.

Standard Medium contents/ liter	Modified Medium contents/ liter
40g Columbia agar base	33.2g Azide blood agar base
10g arabinose	10g arabinose
3.6ml phenol red (2%)	3.6ml phenol red (2%)
75mg aztreonam	0.2g Sodium azide
50 mg Cephalixin	50 mg Cephalixin

All of positive isolates on chromo agar orientation agar we were cultured on this medium. inoculated plates were incubated at 35°C aerobically for 48hrs and examined for growth and fermentation of arabinose. A change in the color of the medium surrounding the colony, from red to yellow indicated arabinose fermentation.

2-Molecular assay

DNA preparation and PCR

A PCR reaction with specific primers was performed to identify *all* isolate belong *E. faecalis* and *E. faecium* as confirmatory test for above result (Table 2). DNA template was prepared as described by (10) 25µl of PCR amplification mixture contained deionized sterile water, 12.5µl Green Go Taq Master Mix pH 8 (Promega,USA) contained [(50unit/ml) of Go Taq DNA polymerase, (400Mm) of each dNTPs and (3mM) of MgCl₂], 1pmol for specific primers (Alpha DNA,Canada). The PCR cycles for *ddl* genes (*ddl E. faecalis* and *E. faecium*) were as followed: initial denaturation at 95°C for 10 min, 30 cycles of denaturation at 94°C for 1min , annealing at 54°C for 1 min and extension at 72°C and final extension at 72°C for 10min using Gradient PCR (TechNet-500, USA).

Table (2).Oligonucleotide primers sequences used for PCR

amplification of *ddl*_{*E. faecium*} ,*ddl*_{*E. faecalis*}

Genes	Sequence (5' to 3')	Size (bp)
<i>ddl</i> <i>E. faecium</i>	F: TTGAGGCAGACCAGATTGACG R: TATGACAGCGACTCCGATTCC	658
<i>ddl</i> <i>E. faecalis</i>	F: CATGAATAGAATAAAAGTTGCAATA R: CCCCTTTAACGCTAATACGATCAA	1030

Result and Discussion

The preliminary result of identification of members of genus of *Enterococcus* revealed that 105 isolates of *Enterococcus* were identify by growing of azid blood agar & bile esculin media as shown in figure (1) and figure (2). The confirmative test used in this paper showed that all isolates

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previously identify as *Enterococcus* by preliminary test were succeeded to procedure turquoise color on chromogenic agar orientation which is indicted for existence of *Enterococcus* species (figure 3) the identify modify media showed the growth of 55(7.8%) isolates of *E. faecalis* and 50 (7.1%) isolates of *E. faecium* as indicated in figure(4) .

this result agreement with (9) which consider the first researcher worked on cephalixin-aztreonam arabinose medium after her Modified . the *E. faecium* change medium color from red to yellow while *E. faecalis* not arabinose fermenter and not change of medium. in this medium showed 50 isolates were *E. faecium* and 55 isolates were *E. faecalis* .

On the other hand the result of PCR amplified technique indicted with no doubt that all isolates previously identification as *Enterococcus* species either faecalis or faecium were amplified used *ddl E. faecium* and *ddl E. faecalis* primer (figure 5&6). The (*ddl_{E. faecium}* *ddl_{E. faecalis}*) genes which is responsible for the constitution of cell wall of *E. faecium* and *E. faecalis* was used, the result) showed that all arabinose fermenting isolates and not fermented gave positive result (clear band) on 1.5% agarose gel as a result of PCR reaction.

the result of this study is beyond the conducting of percentage of infected cause by member of *E. faecalis* yet , the aim of study is to compared between different method of identification to select the best method for this purpose.

The result of this study discard clearly that alled method here is as equal each other in identification member of *Enterococcus* namely faecalis and faecium but PCR amplification technique still the more specific for this purpose(10).

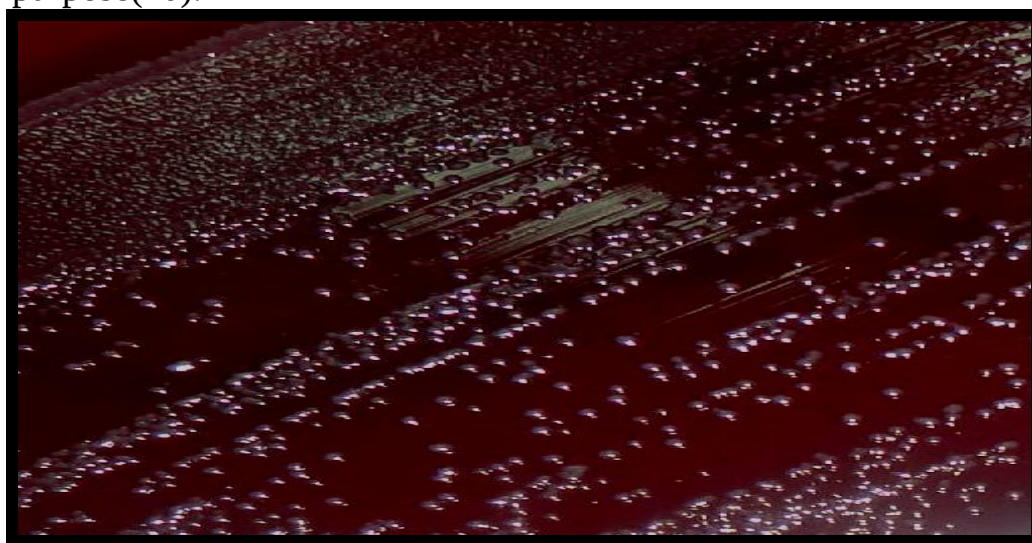


Figure (1): *Enterococcus* on azide blood agar medium incubated aerobically at 35°C for overnight.

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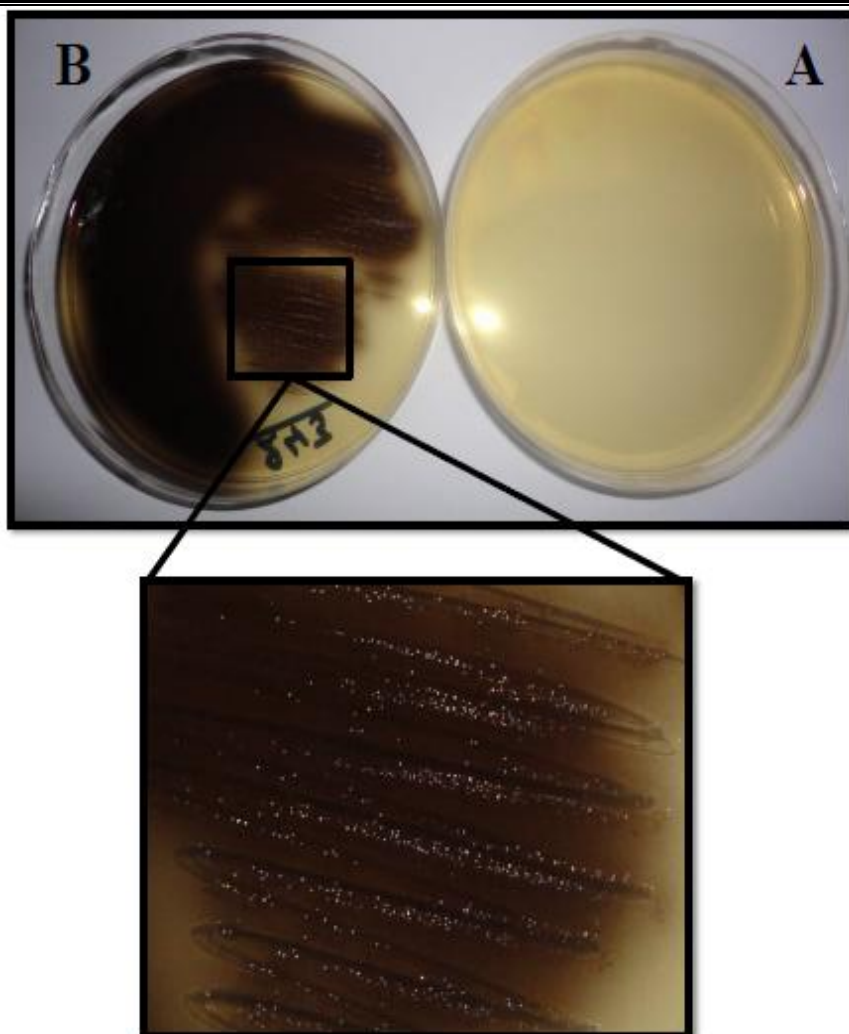


Figure (2): Bile esculin medium. (A) Blank medium without culture.
(B) Esculin hydrolysis by *Enterococcus* species.

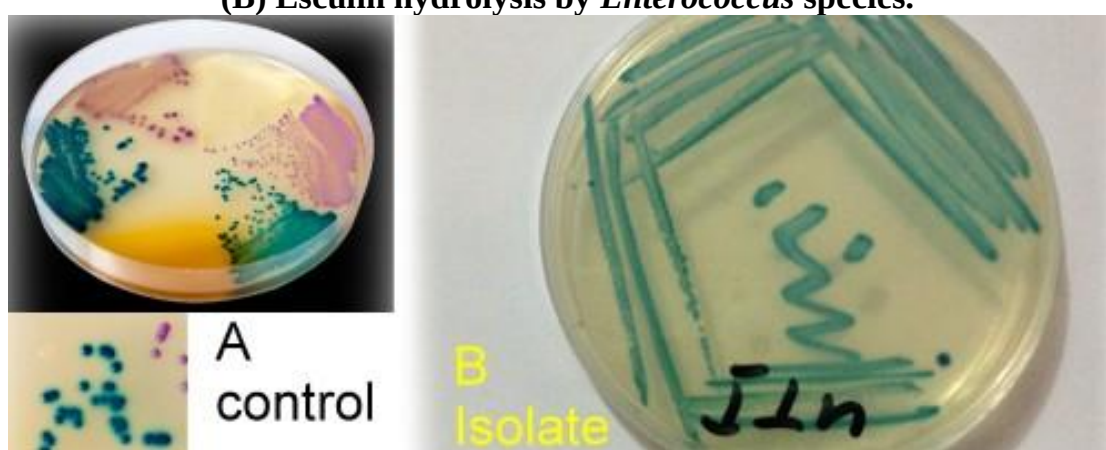


Figure (3) A-Enterococcus control on chromogenic UTI orientation agar by company B-Enterococcus growth on chromogenic UTI orientation agar and colonies trquazi color.

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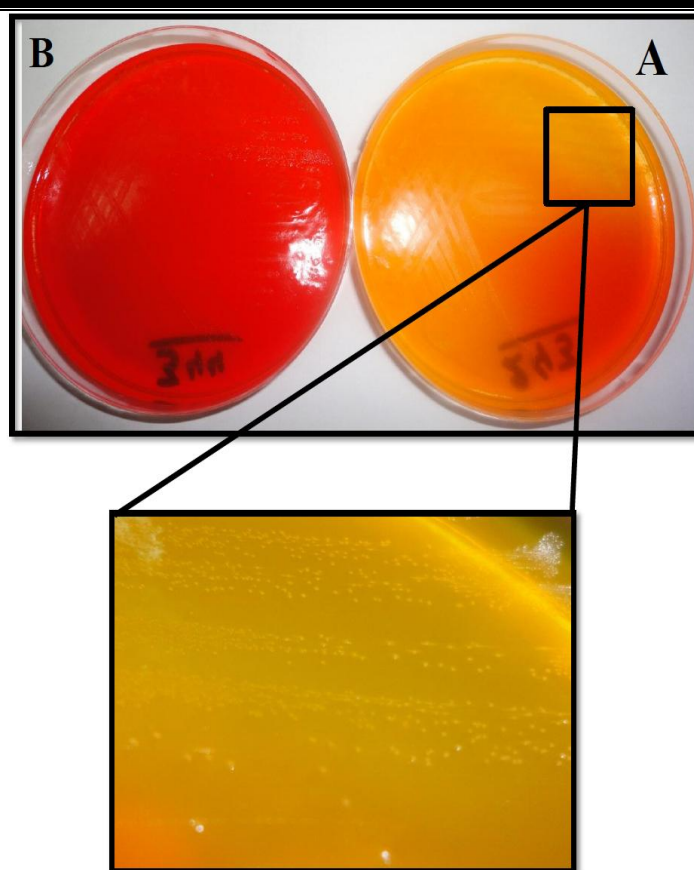


Figure (4): Modified CAA medium (A) Arabinose fermentation by *Enterococcus faecium*. (B) Not arabinose fermentation by *Enterococcus faecalis*.

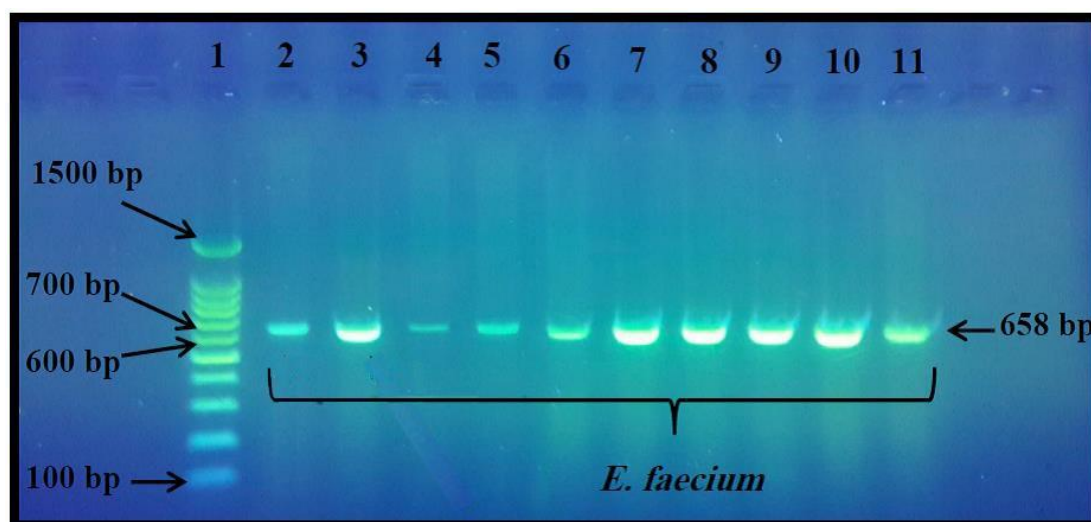


Figure (5): PCR amplification of *ddl_{E. faecium}* .

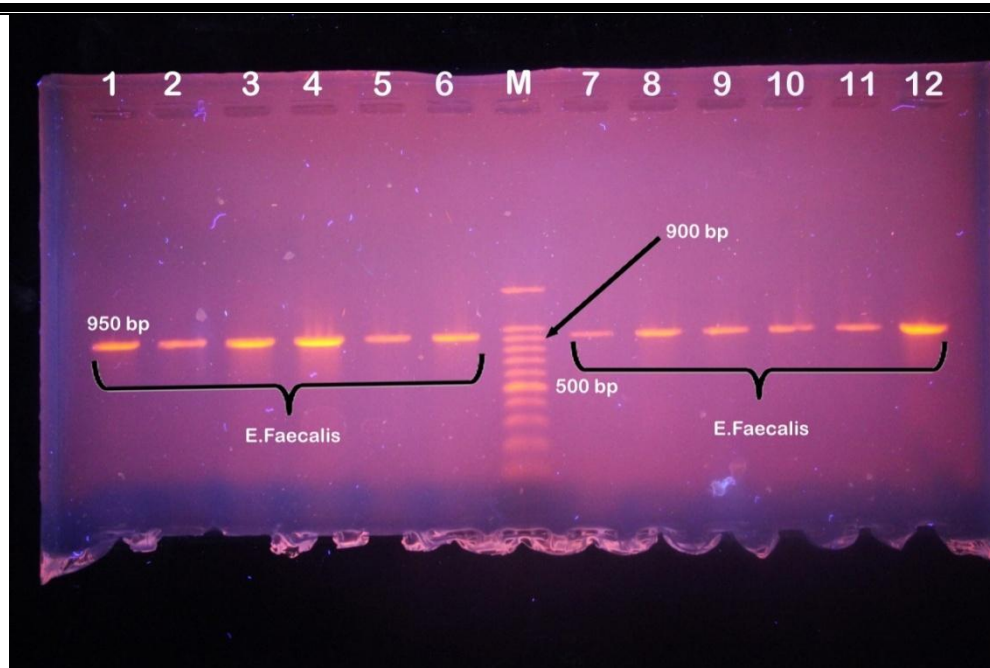


Figure (6): PCR amplification of *ddl_{E. faecalis}* gene.

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