Selective Chromogenic Agar Medium for Detection of Vancomycin-Resistant Enterococcus species

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وَأَنْزَلَ اللهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ
وَعَلَّمَكَ مَا لَمْ تَكُنْ تَعْلَمُ
وَكَانَ فَضْلُ اللهِ عَلَيْكَ عَظِيمًا
صدق الله العظيم
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Contents

Page

Contents *(Cont..)*

INTRODUCTION

Enterococcus species are members of the normal intestinal flora and are the most common aerobic gram-positive cocci found in the large bowel of humans. They may be associated with invasive disease. Common sites of clinical infection include the urinary tract, the blood stream, and intra-abdominal or pelvic wounds ***(Treitman et al., 2005).***

In recent years, their clinical importance has been amplified by an increased resistance to antimicrobials and, in particular, an acquired resistance to glycopeptides first documented in 1988 ***(Cuzon et al., 2008).*** Since this first reported outbreak, vancomycin-resistant *Enterococci* (VRE) has become a common cause of health care associated infection across many parts of the world ***(Top et al., 2008).***

Two clinically relevant species, *Enterococcus faecalis* and *Enterococcus faecium*, have been shown to acquire resistance to glycopeptides and account for a majority of enterococcal infections ***(Treitman et al., 2005).*** Several genes encode resistance to glycopeptides; however Van A and Van B are of greatest concern. Van A is transposon mediated and confers inducible high-level resistance to both vancomycin and teicoplanin, whereas Van B can be plasmid or chromosomally

mediated and confers moderate to high resistance to vancomycin only ***(Courvalin, 2005).***

The clinical impact of infection by vancomycin-resistant *Enterococc*i (VRE) has been examined in several studies, with the most notable consequences being increases in mortality, length of hospital stay, and cost of hospitalization ***(Ledeboer et al., 2007).***

Management of a VRE outbreak requires strategies to contain cases and decrease rates of transmission, including isolation of VRE infected or colonized patients ***(Zirakzadeh and Patel, 2006).*** Early detection of VRE in fecal specimens is important for nosocomial prevention measures, epidemiologic infectious disease follow-up, and also prevention of vancomycin-resistant *Staphylococcus aureus* emergence ***(Delmas et al., 2007).***

There is a continuous need for improvement of culture methods for VRE detection, especially for those clinical microbiology laboratories that do not have access to nucleic acid amplification technologies ***(Paule et al., 2003).*** Recently, chromogenic media incorporating chromogenic enzymatic substrates and a variety of antimicrobial agents (chrom ID VRE, CHROM agar GRE) have become available for VRE detection in one single step, but only Chrom ID VRE containing vancomycin in a concentration of 8μg/ml, can

additionally directly differentiate between vancomycin-resistant *E. faecium* and *E. faecalis* strains from clinical specimens based on distinct colony color ***(Kuch et al., 2009).***

AIM OF THE WORK

The aim of this study is to evaluate the usefulness of chromID VRE agar for rapid isolation of Vancomycin-resistant *Enterococcus* from stool samples or rectal swabs of immunocompromised patients in comparison with modified bile esculin agar.

ENTEROCOCCI

Taxonomy:

The name "*Enterococcus*" is derived from the French word "*Entérocoque*", which was used at the turn of the century to describe the enteric origin of this gram-positive coccus ***(Murray, 2000).***

Enterococci were originally classified as enteric gram-positive cocci and later included in the genus *Streptococcus*. In the 1930s, with the establishment of the Lancefield serological typing system, *Enterococci* were classified as group D *Streptococci* and were differ-rentiated from the nonenterococcal group D *Streptococci* such as *Streptococcus bovis* by distinctive biochemical characteristics. In the 1980s, based on genetic differ-rences, *Enterococci* were removed from the genus *Strep-tococcus* and placed in their own genus, *Enterococcus*. The previously used species designations such as *faecalis, faecium, durans,* and so forth were retained but were preceded by the genus name *Enterococcus* in place of *Streptococcus* ***(Cetinkaya et al., 2000).***

The taxonomy of *Enterococcus* species has undergone considerable change since the mid-1980s. Before the advent and widespread use of genetic techniques for taxonomic analysis, *Enterococci* were distinguished from *Streptococci*

and related taxa by the ability to grow at 10°C and 45°C, growth in the presence of 6.5%NaCl, and at pH 9.6, ability to hydrolyze esculin in the presence of 40% bile, and production of pyrrolidonyl arylamidase (PYR). More than 90% of strains also contained the Lancefield group D lipoteichoic antigen in their cell wall. Taxo-nomic studies have subsequently revealed species that are members of the genus *Enterococcus* by genetic criteria, but that lack many of the phenotypic charac-teristics typical of the genus. Fortunately, most of these species are not commonly found in human clinical specimens ***(Koneman et al., 2006a)*.**

Natural habitat:

Several intrinsic characteristics of the *Enterococci* allow them to grow and survive in harsh environment and to persist almost everywhere ***(Teixeira et al., 2007)***. *Enterococci* can be found in soil, on plants, in milk products, other foods and in high numbers, as part of the normal microflora in the gastrointestinal tract as well as the faeces of vertebrates ***(Domig et al., 2003b).*** But they may also colonize the upper respiratory tracts, biliary tracts, and vaginas of otherwise healthy persons. The isolation of clinical isolates of *Enterococci* generally denotes colonization rather than infection ***(Zhanel et al., 2001).***

The prevalence of the different enterococcal species appears to vary according to the host and is also influenced by age, diet, and other factors that may be related to changes in the physiologic conditions ***(Teixeira et al., 2007)***.

Enterococcal species:

Enterococcus faecalis and *Enterococcus faecium* are the predominant enterococcal species identified in clinical microbiology laboratories. Historically, these laboratories report that 80 to 90% of *Enterococci* are *E. faecalis*, whereas *E. faecium* accounts for 5 to 10% of *Enterococci*. The *E. durans, E. avium, E. raffinsus, E. gallinarum, and E. casseliflavus* are reported much less frequently than *E. faecalis* and *E. faecium* ***(Chaudhary et al., 2007)*.**

Over the past few years, several new entero-coccal species have been described from human clinical sources (i.e., *E.gilvus, E.pallens*, CDC PNS [for probable new species]-E1, CDC PNS-E2, CDC PNS-E3), animals (i.e., *E.canis*, *E.villorum* [same as *E.porcinus*], *E.ratti*, *E.asini*, *E.phoeniculicola*), and the environment (i.e., *E.haemoperoxidus*, *E.moraviensis*). Based on their phenotypic characteristics, the *Enterococci* are divided into 5 phenotypic groups (Table 1). With the help of molecular taxonomic techniques, some former *Enterococcus* species have been reclassified or found to be the same as previously recognized species. For example, *E.seriolicida* was found to be

identical to *Lactococcus garvieae*, *E.flavescens* was shown to be the same as *E.casseliflavus*, and *E.solitaries* was to be identical to *Tetragenococcus halophilus* ***(Koneman et al., 2006a).***

Table (1): Different enterococcal species ***(Koneman et al., 2006a)***

GROUP/Species	Comments
GROUP1	
E.avium	Isolated from avian, canine, and human gastrointestinal tracts; strains may carry both Lancefield group D and group Q carbohydrate antigens; this species (and *E.malodoratus*) are the two enterococcal species that produce H_2S. *E. avium* has been isolated from cases of bacteremia and osteomyelitis.
E.gilvus	New species described in 2002; originally isolated from a bile specimen of a patient with cholecystitis.
E.malodoratus	Isolated from Gouda cheese and unpasteurized milk products; name means ill "smelling''; also produces H_2S.
E.pallens	New species described in 2002; isolated from a peritoneal dialysate specimen of a patient in whom peritonitis developed from a perforated intestine.
E.pseudoavium	Genetic relatedness studies and certain phenotypic characteristics differentiate this species from *E. avium*; type strain isolated from a case of bovine mastitis.
E.raffinosus	Originally considered to be related to *E. avium* (along with *E. solitaries* and *E. pseudoavium*); named for its ability to produce acid from raffinose; recovered from human infections, including blood cultures, urine, abscesses, and vertebral osteomyelitis.
E.saccharolyticus	Originally called *Streptococcus saccharolyticus*; described a group of *S. bovis*-like strains recovered from cows; genetic analysis showed that this organisms is more closely related to the *Enterococci* than the *Streptococci*; has certain phenotypic characteristics that are similar to *Enterococcus* species (eg., growth at 10 ^{O}C and 45 ^{O}C, growth in 6.5%NaCl), but does not react with group D antisera; proposed that this species be moved from the viridians *Streptococcus* group to the genus *Enterococcus* as *E. saccharolyticus.*
Enterococcus sp. CDC PNS-E-3	CDC "proposed new species," isolated from brain tissue obtained from an 11-month-old patient in Honolulu, Hawaii, in 2001.
GROUP 2	
E.faecalis	Most frequent isolate from human specimens and from the human gastrointestinal tract; also found in the intestinal tracts of poultry, cattle, pigs, dogs, horses, sheep, and goats.
E.faecium	Found in human clinical specimens; generally more resistant to antimicrobial agents than *E. faecalis*; also found in the gastrointestinal tracts of various species of animals.
E.casseliflavus	Recovered from plants, soil, and rarely, from the feces of chickens; originally classified as a subspecies of *E. faecium*; produces a yellow pigment and is also motile. This organism is an opportunistic agent in human infections and has been isolated from patients with bacteremia.
E.gallinarum	Isolated from chicken feces; originally classified as *S. gallinarum* "chicken group D"; one of the two motile *Enterococcus* species; has also been isolated from an infection in a hemodialysis patient, as a cause of native-valve endocarditis, and from blood cultures.
E.mundtii	Yellow-pigmented non motile organism; isolated from plants, soil, and the gastrointestinal tracts of cattle, pigs, and horses; named after J.O. Mundt, an

GROUP/Species	Comments
	American microbiologist. This species has been isolated from a human thigh abscess and from an operatively obtained sinus mucosal specimen.
E.haemoperoxidus	New environmental enterococcal species described in 2001; isolated from surface waters, swimming pools, and drinking water in the North Moravia region of the Czech Republic.
Enterococcus sp. CDC PNS-E2	CDC "proposed new species," isolated from blood cultures of a patient in Los Angeles in 1997
GROUP 3	
E.dispar	Species originally thought to be a biochemical variant of *E. hirae*, but analysis of 16S rRNA indicated that this organism is indeed a previously undescribed species; recovered from human specimens, including stool and synovial fluid.
E.durans	This species is found mainly in milk and other dairy products and is a rare clinical isolate. In 2004 *E. durans* was isolated as a cause of native-valve endocarditis
E.hirae	Causes growth depression in chickens; isolated from chicken drops and feces, and the gastrointestinal tracts of cattle, pigs, dogs, horses, sheep, goats, and rabbits; type strain (*E. hirae* ATCC 8043) has complex nutritional requirements and is used in the food industry as a bioassay organism for amino acids and vitamins. *E. hirae* was isolated from the blood of a 49-year-old patient with end-stage renal disease who was undergoing hemodialysis.
E.ratti	Described in 2001, this new species was originally isolated from the intestines and feces of rats with diarrhea.
E.villorum	Isolated from the gastrointestinal tract of dogs; phenotypically identical with *E. porcinus*, which is considered as a junior synonym of *E. villorum* and was originally isolated from the intestines and feces of pigs with diarrhea.
GROUP 4	
E.asini	Described in 1998, this species is found in the cecum of donkeys; most closely related to *E. avium*, *E. pseudoavium*, and *E. faecium*.
E.cecorum	Newly reclassified streptococcal species found in the intestines of chickens; lacks the group D antigen, is PYR-negative and is unable to grow in 6.5% NaCl broth; similar to *E. columbae*, it may be confused with *E. avium* and with *S. bovis*. *E. cecorum* has been isolated as a cause of peritoneal dialysis-associated peritonitis, recurrent bacteremic peritonitis, and spontaneous bacterial peritonitis with empyema, and has been isolated from blood cultures of a patient with severe malnutrition.
E.sulfureus	Newly described yellow-pigmented species; recovered from plants; has not yet been isolated from humans.
E.phoeniculicola	Described in 2003, this species is found in the preen glands of wild red-billed Woodhoopoes; does not grow on BE agar or in 6.5% NaCl broth.
Enterococcus sp. CDC PNS-E1	CDC "proposed new species," isolated from the blood of a patient in Evanston, Illinois, in 1991
GROUP 5	
E.columbae	Newly reclassified streptococcal species isolated from the intestinal tract of pigeons; closely related to *E. cecorum* and *E. avium*; characterized by being PYR-negative and unable to grow in 6.5% NaCl broth.
E.canis	Originally isolated from a dog with chronic otitis externa; probably a resident