Isolation of yeasts from Otomycosis cases in children and dogs

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ABSTRACT

Otomycosis usually unilateral with scaling, itching, and pain as the primary symptoms. The infection is either subacute or acute. This research was conducted to isolate yeasts from otomycosis in children and dogs in Baghdad city. Ear swabs from 100 child which were diagnosed clinically in central educational hospital of pediatrics and 100 dog's cases were brought to private clinics with otitis symptoms and subjected to fungal isolation by macroscopic and microscopic methods by using RapID Yeast Plus System for yeasts identification. The results for yeasts isolation from ear swabs of children suffered from ear infections were thirty four (34) yeasts isolates (34%), in which C. albicans appeared as highly occurrence in ten (10) isolates (10%) whereas Cr. albidus showed nine (9) isolates (9%), C. tropicalis with eight (8) isolates (8%), C. lusitaniae with three (3) isolates (3%) as well as C. krusei and C. intermedia appeared with two (2) isolates (2%) for each one of them, while the results of yeasts isolation from dogs ear swabs suffered from problems in ears were sixteen (16) yeasts isolates (16%), C. albicans represent highly appeared species with five (5) isolates (5%) while C. glabrata appeared with three (3) isolates (3%) whereas Cr. albidus and R. rubra showed four (4) isolate (4%) for each of them.

Key word: Yeast, children, dogs, otomycosis, C.albicans, RapID Yeast Plus System


Introduction

Otomycosis is fungal infection of the external auditory meatus and ear canal, the disease may extend in the middle ear if the tympanic membrane is perforated, the hot and humid environment of tropics and subtropics makes otomycosis more prevalent in these regions (Fasunla et al. 2008). Multiple and prolonged use of broad-spectrum antibiotics, trauma, persistent otorrhoea and swimming have been documented as predisposing factors (Rutt and Sataloff, 2008; Vennewald and Klemm, 2010). Wide variety of fungi have been implicated in the causation of mycotic infection of the ear; the most common organisms include Aspergillus and Candida species (Aneja, et al. 2010; Viswanatha and Naseeruddin, 2011). The main risk factors for otomycosis include moisture; minor inflammation; the use of broad spectrum antibiotics, steroids, chemotherapeutic agents, or topical ear drops; physical injury; living in warm and humid climates; and frequent bathing or swimming (Wang et al. 2005; Yavo et al. 2004). In addition, immunocompromised hosts are more vulnerable to otomycosis. Patients with diabetes, lymphoma, HIV, endocrine abnormalities, changes in hormonal balance, or a history of transplantation, as well as patients receiving chemotherapy or radiotherapy, are at a greater risk of contracting otomycosis (Bhally et al. 2004; Fasunla et al. 2008).
Materials and Methods

Two hundred (200) ear swabs sample had been collected from children and dogs, one hundred (100) samples were from children cases aged between (4-16) years old in Educational Central Hospital for Pediatrics and one hundred (100) samples were from dogs came with their owners to the private clinics, hence in both cases (children and dogs) they were suffered from disease in the ear region, the collection had been done by using sterile cotton swabs and after disinfect the external ear and surrounding skin with 70% ethanol alcohol and after drying it, sterile cotton swabs was used in taking the samples from the inner parts of ear and then put it directly on to sterile brain heart infusion broth tubes incubated in 30 Cº for 1-2 weeks, if the suspected growth appear on this primitive medium, its cultured on Sabouroid dextrose agar incubated at 30 Cº for 1-2 weeks, the confirmatory tests had been done on suspected yeasts growth by using RapID™ yeast plus system (1) (Fig 1).

Culture media:

**Sabouraud dextrose agar:** The medium was prepared by dissolving 56 gm of media in 1000 ml of distilled water. After autoclaving at 15 lbs pressure / 121ºC for 15 minutes the media temperature was lowered up to 50ºC & chloramphenicol 0.05 gm /lit was added to the media which was used for isolation

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Brain heart infusion broth:

This medium was prepared by suspended 37 gm of media in 1000 ml of distilled water after boiling and dissolving chloramphenicol 0.05 gm/lit was added to prevent bacterial growth then it was distributed into sterile glass covered tubes, autoclaving at 15 lbs pressure / 121ºC for 15 minutes and kept in the refrigerator for primary isolation from ear swabs.

Identification tests:

RapID™ yeast plus system

Constituents of the Kit

1. RapID yeast plus panels.
2. RapID yeast plus reagent A.
3. RapID yeast plus reagent B.
4. RapID inoculation fluid (2 ml/ tubes)

Each panel has several reaction cavities which contain dehydrated reactants and the tray allows the simultaneous inoculation of each cavity with determined amount of inoculums

A suspension of the test organism in RapID inoculation fluid is used as the inoculum which rehydrates and initiates test reactions, after incubation of the panel each test cavity is examined for reactivity by noting the development of color. In some cases, reagents must be added to the test cavities to provide a color change.

The resulting pattern of positive and negative test scores was used as the basis for identification of the tested isolates by comparison of test results to reactivity patterns stored in a database or through the use of a computer generated code compendium. So the tests used in this system are based on microbial degradation of specific substrates detected by various indicator system.

RapID™ yeast plus system protocols

1. Test organisms had been grown in pure culture on Sabouraud dextrose agar and examined by gram stain or wet mount prior to use in the system so that only organisms which demonstrate yeast-like appearance and growth characteristics were tested by using the RapID yeast plus system.
2. Cultures used for inoculum preparation incubated at 30C for 48hours old.
   - By using inoculating loop, suspend sufficient growth from the agar plate culture in the inoculation fluid (2mls) to achieve visual turbidity which will done by select well-isolated colonies of the test isolate and add to the inoculation fluid to avoid clumping and over-inoculation, continue to add organism until the turbidity of the suspension completely obliterates the black lines on the inoculation card, once the black lines on the
inoculation card are no longer visible, inoculum preparation is complete, then suspension should be mixed thoroughly and sometimes the suspension was vortexed and then used within 15 minutes of preparation.

**Inoculation of RapID™ yeast plus panels:**

1. The lid of the panel over the inoculation part had been peeled back.
   - The entire contents of the inoculation fluid had been transferred gently by a pipette in to the upper right-hand corner of the panel, and then reseal the inoculation part of the panel; the inoculated panels were incubated at 30°C incubator for 4 hrs.

**Scoring of RapID™ yeast plus panels:**

These panels contain 18 reaction cavities that provide 18 test scores. The reagent requiring tests (cavities 7-14 and 16-18) were designated with a box drawn around them.

A. One drop from reagent A to cavities 7 through 14.
B. One drop of reagent B to cavities 16 through 18.

1. After the addition of both reagents we allow 30 second-1 minute for the color development.
2. Read and scored the test cavities from left to right by using the interpreting guide.
3. Reference the micro code obtained on the report form in the RapID yeast plus code compendium or ERIC for the identification.

**Results and Discussion**

The results of yeasts isolation from ear swabs of children suffered from ear infection were 34%, appeared as thirty four (34) isolates, in which the *C. albicans* appeared as highly occurrence in ten isolates (10%) whereas *Cr. albidus* showed nine isolates (9%), *C. tropicalis* with eight isolates (8%), *C. lusitaniae* with three isolates (3%) as well as *C. krusei* and *C. intermedia* appeared with two isolate (2%) for each one of them (Table 1).

<table>
<thead>
<tr>
<th>Yeast Species</th>
<th>No</th>
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<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>10(10%)</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>8(8%)</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>2(2%)</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>3(3%)</td>
</tr>
<tr>
<td><em>C. intermedia</em></td>
<td>2(2%)</td>
</tr>
<tr>
<td><em>Cr. albidus</em></td>
<td>9(9%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>34(34%)</td>
</tr>
</tbody>
</table>

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The results of yeasts isolation from ear swabs of dogs revealed sixteen isolates with (16%) , C. albicans appeared with five isolates (5%) , Cr. albidus with four isolates (4%), whereas C. glabrata with three isolates (3%) and R. rubra scored four isolates (4%).(Table 2)

Table (2) yeasts isolates from dogs ear swabs samples

<table>
<thead>
<tr>
<th>Yeast Species</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans</td>
<td>5(5%)</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>3(3%)</td>
</tr>
<tr>
<td>Cr. albidus</td>
<td>4(4%)</td>
</tr>
<tr>
<td>R. rubra</td>
<td>4(4%)</td>
</tr>
<tr>
<td>Total</td>
<td>16(16%)</td>
</tr>
</tbody>
</table>

This research approved appearance of non–albicans Candida species such as Candida tropicalis , Candida lusitaniae , Candida krusei , Candida intermedia and this is agree with Del Castillo et.al.1997 who reported that an increased frequency of infections by non-albicans Candida species and the conventional methods for the identification of Candida species which were based on assimilation, fermentation reactions, were potentially reliable.

Hence the yeasts diagnosis in this research were depended upon RapID™ yeast plus system Fig(2) which is qualitative micro method employing conventional and chromogenic substrates for the identification of medically important yeast, yeast-like and related organisms isolated from clinical specimens and this is agree with Mirhendi et.al.2008

Fig(2): Panels of RapID™ yeast plus system after inoculation with tested yeasts, so the 4 panel represented the result for C.krusei and the 5 panel represented the result for C. albicans while the 6 panel represented the result for C. lusitaniae.

So the sensitivity and accuracy of RapID™ yeast plus system was very high as showed by Hossein et.al.2011 when compared between the molecular (RFLP-PCR) and enzymatic methods (RapID™ Yeast Plus System) and demonstrated that both of them have a similar sensitivity to the Candida species and similar to that was Smith et.al.1999 when Compared the Performance of the RapID™ Yeast Plus System and the API 20C AUX system in the diagnosis of clinical yeasts isolates and found there was 97.3% correlation between these two system ,as well as Kitch et.al.1996 who found that ,the accuracy of RapID™ Yeast Plus System was 94.1% in identification of yeasts isolates tested
The highly prevalence of otomycosis mostly due *Candida albicans* and non–albicans species has been linked to extensive use of ototopical fluoroquinolones which is established by Jackman et al. 2005

The results in this research was conducted with Mahmoudabadi et al. 2009 in Iran who able to isolate *C. albicans* in (5.9%) children which were used hearing aids, and with Saki et al. 2013 in south-west Iran which were isolated *Candida albicans* in (11.6%) from 293 cases of otomycosis in addition to results established by Arsovíc et al. 2009 in Belgrade were recovered *C. albicans*, *C. parapsilosis*, *C. guilliermondii*, and *C. famata* from 23 children suffering from otomycosis, from the bony portion of the external ear, as well as Pontes et al. 2009 in Brazil established that the most frequently isolated species from otomycosis were *C. albicans* (30%), so the results in the present research were similar to what established by Martin et al. 2005 in that the fungal causes of otitis externa and tympanostomy tube otorrhea constitute 72% of 166 children were suffered from otitis externa and otitis media from which *Candida albicans* was identified in 43% of fungal cultures which is agree with Pundir et al. 2007 and Janos, 2009 which were suggested that *Candida species* represented the most common isolated yeasts of the infectious otitis

This research established *Cr. Albidus* as the main causative agent in nine cases of children and four cases of dogs which is comparable to a survey done by Bernardo et al. 1998 on mycotic otitis externa in which *Cryptococcus species* appeared in (13.1%) from positive cases.

References:


