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Resistance Problem of Coryneform Bacteria Isolated from Intensive Care Unit Samples*

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Abstract: In this study the importance of the need to take coryneform bacteria as opportunistic pathogens of the respiratory tract, especially in ICU patients, into consideration was emphasized. Coryneform bacteria isolated from respiratory specimens of Intensive Care Unit (ICU) patients in hospital were identified to the species level and their antimicrobial susceptibilities were performed over a period of sixteen months, between January 2002 and April 2003. The identifications of isolated bacteria were done with API Coryne (Bio Merieux, France) identification system. Twenty Coryneform bacteria were isolated from respiratory specimens; 10 (50%) were *Corynebacterium striatum,* 5 (25%) *C. amycolatum*, 3 (15%) *C. pseudodiphtheriticum* and 2 (10%) *C. accolens*. Antimicrobial susceptibilities of these strains against 16 antibiotics showed that all isolates were sensitive to vancomycin and teicoplanin, but *C. amycolatum* strains were multiply resistant to most of the antimicrobial agents. Thus emerging antimicrobial resistance in various species has created an additional need for accurate identification of coryneform organisms at the species level and continuous surveillance of their resistance patterns. In this study the importance of the need to take coryneform bacteria into consideration especially as pathogens of opportunistic infections in ICU patients and the importance of assessing their antibiotic sensitivity is emphasized.

Key words: Coryneform bacteria, antimicrobial susceptibility testing, respiratory spceimeus, ICU

INTRODUCTION

Corynebacterium species are widely distributed and are members of skin and the mucous membrane (Coyle and Lipsky, 1990; Clarridge and Spiegel, 1995; Riegel *et al*., 1996). Corynebacteria other than *Corynebacterium diphtheria* have since been referred to as diphtheroids and coryneforms and have been considered colonizers and contaminants (Funke *et al.,* 1997; Brown, 2000). After long lasting discussion and confusion about their clinical significance, coryneforms have emerged as important pathogens. Noteworthy are the increased numbers of opportunistic infections due to coryneform bacteria as the numbers and survival of severely immunocompromised patients increase and the numbers and types of medical devices used in both immunocompromised and immunocompetent patients increase. These organisms are reported to cause serious infections such as;

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bacteremia, valvular endocarditis, neurosurgical shunt infection, meningitis, brain abscess, peritonitis, osteomyelitis, septic arthritis, pneumonia, empyema and urinary tract infections (Brown, 2000).

Identification of coryneform bacteria to the species level often causes problems (Funke *et al*., 1997). Coyle and Lipsky (1990) clearly stated that the recognition of infections caused by coryneform bacteria is highly dependent on the laboratorian's ability to identify these species. In 1981 Hollis and Weaver were the first to systematically examine the full range of coryneform bacteria isolated from clinical specimens. Their guide for the identification of gram-positive rods turned out to be an invaluable source for clinical microbiologists aiming to identify clinical isolates and became the basis for later taxonomic investigations on coryneform bacteria (Funke *et al.,* 1997). Also, numerous researchers have made suggestions related to this subject. Clarridge's and Spiegel (1995) and Funke *et al.* (1997) published a comprehensive study regarding the identification and susceptibility testing of coryneform bacteria.

The aim of this study was the identification and antimicrobial susceptibility testing of coryneform bacteria isolated from respiratory specimens of patients in ICU.

MATERIALS AND METHODS

The coryneform bacteria were collected over a period of sixteen months from January 2002 to April 2003. A total of 594 respiratory specimens were routinely submitted for culture to the microbiology laboratory from ICU department of the University Hospital of Gaziantep located in the South-eastern part of Turkey, a 400-bed teaching hospital. The specimens were transported via Stuart's transport medium to the laboratory. They were cultured on blood agar supplemented with 5% sheep blood and incubated at 37°C in 5% CO_2-enriched conditions for 24-48 h. The bacteria that depicted coryneform bacteria morphology as pleomorphic bacilli representing V forms or Chinese letters with Gram stain were accepted as significant when found in pure culture in normally sterile sites or as predominant organism in nonsterile sites of the respiratory tract. In addition to Gram staining, other differentiation procedures such as colony morphology, pigmentation, motility, catalase, oxidase reaction, nitrate reduction, urea hydrolysis and CAMP test were performed. Data were compared with identification schemes of Funke *et al.* (1997).

Strains which yielded coryneform bacilli with conventional methods, were identified to the species level with API coryne system (Bio Merieux, France). Strains identified as being *C. striatum/C. amycolatum* with API Coryne system were further tested for tyrosine hydrolysis which was positive for *C. striatum*, but negative for *C. amycolatum* (Martinez-Martinez *et al*., 1995; Martinez-Martinez *et al*., 1996; Riegel *et al.,* 1996; Funke *et al*., 1997; Lagrou *et al*., 1998). Another criteria which is useful in distinguishing these species is the colony morphology. As *C. amycolatum* colonies appear yellowish, dry and granular on sheep blood agar, *C. striatum* colonies are whitish, moist and smooth (Funke *et al*., 1997).

So far, the National Committees for Clinical Laboratory Standards (NCCLS) has not yet published a suggestion related to antimicrobial susceptibility test for *Corynebacteria* (Clarridge and Spiegel, 1995; Funke *et al*., 1997; Ustaçelebi, 1999). At present, it is not clear which culture medium should be used for growing the inoculum for antimicrobial susceptibility testing, which medium is to be used for broth microdilution or agar dilution techniques, or which incubation conditions are appropriate for use. In some studies, the NCCLS categories for *staphylococci* have been applied when testing coryneforms for susceptibility to penicillin, whereas others recommend the use of

Streptococcus interpretative criteria when assessing the activity of penicillin against coryneform bacteria (Funke *et al*., 1997). In conducted study ATB STAPH 5 (susceptibility test for *staphylococci*) (Bio Merieux, France) was used for antimicrobial susceptibility testing of coryneform bacteria (Courvalin *et al*., 1985; Martinez-Martinezl *et al*., 1995; Funke *et al.,* 1996; Lagrou *et al.,* 1998). This antimicrobial test comprises 16 antibiotics with following concentrations: penicillin (0.12 mg L^{-1}), oxacillin (2 mg L^{-1}), cephalothin (8 and 16 mg L^{-1}), ampicillin-sulbactam (8/4 and 16/8 mg L^{-1}), gentamicin (4 and 8 mg L^{-1}), netilmicin (8 and 16 mg L^{-1}), erythromycin (0.5 and 4 mg L^{-1}), clindamycin (0.5 and 2 mg L^{-1}), pefloxacin (2 and 4 mg L^{-1}), ciprofloxacin (1 and 2 mg L^{-1}), tetracyclin (4 and 8 mg L^{-1}), cotrimoxazol (2/38 mg L^{-1}), nitrofurantoin (32 mg L^{-1}), rifampicin (1 and 2 mg L^{-1}), vancomycin (4 and 16 mg L^{-1}) and teicoplanin (8 and 16 mg L^{-1}). The results were obtained as resistant, intermediate and sensitive.

Staphylococcus aureus ATCC 25923 was used as control organism.

RESULTS

A total of 594 respiratory specimens from the patients of ICU were sent to the laboratory for bacterial culture. In 20 of these specimens (12 bronchoalveolar washing, 4 tracheal aspirates, 4 pleural fluids) coryneform bacteria grew as pure culture/predominant organisms. These bacteria were subjected to API Coryne identification test and 10 (50%) were identified as *C. striatum,* 5 (25%) as *C. amycolatum*, 3 (15%) as *C. pseudodiphtheriticum* and 2 (10%) as *C. accolens.* Each of the strains were isolated from different patients. In 4 of the cultures coryneform bacteria were isolated as the only pathogen, whereas in 9 of the cultures *Pseudomonas aeruginosa,* in one *Acinetobacter baumannii,* in one *Enterobacter aerogenes* and in one *Klebsiella pneumoniae* grew together with coryneform bacteria. In 4 of the cultures coryneform bacteria were found both with *P. aeruginosa* and *S. aureus.*

Antimicrobial susceptibilities of these strains against 16 antibiotics showed that all isolates were sensitive to vancomycin, teicoplanin and except *C. amycolatum* to ampicillin-sulbactam, which strains were multiply resistant to most of the antibiotics too (Table 1).

Antimicrobial susceptibility tests showed that, 90% of *C. striatum* isolates were sensitive to netilmicin, 80% sensitive to cephalothin and gentamicin, 60% sensitive to penicillin, oxacillin,

Table 1: Antimicrobial susceptibility results of coryneform bacteria

Antibiotic	*C. striatum* (%)	*C. amycolatum* (%)	*C. pseudodiphtheriticum* (%)	*C. accolens* (%)
PEN	60.0	40.0	66.6	50.0
OXA	60.0	40.0	100.0	50.0
CFT	80.0	40.0	100.0	100.0
FAM	100.0	40.0	100.0	100.0
GEN	80.0	40.0	100.0	100.0
NET	90.0	60.0	100.0	100.0
ERY	50.0	20.0	33.3	50.0
CLI	10.0	00.0	00.0	50.0
PEF	00.0	20.0	00.0	50.0
CIP	60.0	40.0	100.0	100.0
TET	30.0	60.0	100.0	100.0
TSU	10.0	40.0	33.3	50.0
FUR	00.0	00.0	33.3	50.0
RFA	50.0	60.0	100.0	100.0
VAN	100.0	100.0	100.0	100.0
TEC	100.0	100.0	100.0	100.0

PEN, Penicillin; OXA, Oxacillin; CFT, Cephalothin; FAM, Ampicillin-Sulbactam; GEN, Gentamicin; NET, Netilmicin; ERY, Erythromycin; CLI, Clindamycin; PEF, Pefloxacin; CIP, Ciprofloxacin; TET, Tetracycline; TSU, Cotrimoxazol; FUR, Nitrofurantoin; RFA, Rifampicin; VAN, Vancomycin; TEC, Teicoplanin

ciprofloxacin and 50% to rifampicin. Isolates of *C. amycolatum* were 60% sensitive to rifampicin and 40% sensitive to penicillin, oxacilline, cephalothin, ampicillin-sulbactam, gentamisine, netilmicin and ciprofloxacin. Isolates of *C. pseudodiphtheriticum* were only 66% sensitive to penicillin, 33% to erythromycin and all isolates were resistant to clindamycin. *Corynebacterium accolens* isolates were 50% sensitive to penicillin, oxacillin, erythromycin and clindamycin.

DISCUSSION

Corynebacterium striatum, C. xerosis, C. minutissimum and C. pseudodiphtheriticum are natural members of pharynx, nose and skin flora. However, these microorganisms are considered to colonize asymptomatically after intensive use of antibiotics and *C. striatum* has been reported to be the cause of human infections with an increasing ratio. These microorganisms have been demonstrated as the cause of bacteremia, fatal pleuro pulmonary infection, catheter-related bacteremia in neutropenic patients with cancer, native valve endokarditis, pacemaker-related endocarditis, meningitis, cerebrospinal fluid shunt infections in children, purulent conjunctivitis, chorioamnionitis, peritonitis and empyema (Coyle and Lipsky, 1990; Markowitz and Coudron, 1990; Van Bosterhaut *et al.,* 1992; Watkins *et al.,* 1993; Brandenburg *et al*., 1996; Weiss *et al*., 1996; Funke *et al.,* 1997; Diculencu *et al*., 1998; Lagrou *et al*., 1998; Brown, 2000).

Corynebacterium striatum is primarily known as to cause respiratory tract and blood infections (Weiss *et al*., 1996). In this study, *C. striatum* strains make up to 50% of the coryneform bacteria isolated from tracheal aspiration cultures. In a similar study by Riegel *et al.* (1996), 31(65%) out of 48 *C. striatum* strains were recovered from respiratory tract. Brandenburg *et al.* (1996) isolated *C. striatum* from patients with serious nosocomial infections and regarded it as the cause of nosocomial infections. Leonard *et al.* (1994) identified an epidemic caused by *C. striatum* comprising of 18 patients and 66.7% of the specimens were sputum.

In our study 25% coryneform bacteria were identified to be *C. amycolatum* and 15% *C. pseudodiphtheriticum*. *Corynebacterium amycolatum* has been reported first in 1997 as the causative agent of a neonatal septicemia (Berner *et al.,* 1997; Ustaçelebi, 1999) and than again in septicemia of two leukemia patients (De Miguel *et al.,* 1999). Corynebacterium pseudodiphtheriticum is a member of the oropharyngeal flora and is reported to be responsible for respiratory infections such as pneumonia and bronchitis in immunosuppressed patients. Endotracheal intubation and inhibition of cough reflex are risk factors for those infections (Manzella *et al*., 1995; Funke *et al*., 1997; Ustaçelebi, 1999).

Corynebacterium accolens is usually isolated from wound drainage and endocervical and respiratory specimens. In this study 10% of isolates was identified as *C. accolens*.

The National Committee for Clinical Laboratory Standards (NCCLS) has not published specific guidelines for susceptibility testing of coryneform bacteria so far. This may reflect the underrecognition of underestimation of coryneform bacteria isolated from clinical specimens. It is not clear which medium should be used for growing the inoculum for susceptibility testing, or which incubation conditions should be used. In a pragmatic approach, the NCCLS categories for staphylococci have been applied when testing coryneforms for susceptibility to penicillin (Clarridge and Spiegel, 1995; Funke *et al.,* 1997). In the present study this method was used also.

According to the literature, *C. striatum* is sensitive to penicilin G and β-lactam antibiotics and is resistant mostly to ciprofloxacin, erythromycin, rifampicin and tetracycline. No vancomycin-resistant strain has been reported (Brown, 2000). In this study all *C. striatum* isolates were found to be sensitive to vancomycin, teicoplanin and ampicillin-sulbactam, whereas they were 60% sensitive to penicillin, oxacillin, ciprofloxacin and 50% to rifampicin and erythromycin. Riegel *et al.* (1996)

in their study reported that *C. striatum* isolates were 100% sensitive to ampicillin and cefotaxime, 81% to gentamicin, 75% to ciprofloxacin, 63% to erythromycin and 50% to rifampicin. Brandenburg *et al.* (1996) reported *C. striatum* strains to be susceptible to penicillin, amoxicillin, erythromycin and vancomycin, but resistant to sulphonamides, clindamycin, tetracycline, rifampicin and chloramphenicol.

Isolates of *C. amycolatum* were 60% sensitive to rifampicin and 40% sensitive to penicillin, oxacilline, cephalothin, ampicillin-sulbactam, gentamisine, netilmicin and ciprofloxacin, which were consistent with the literature (Riegel *et al*., 1996; Lagrou *et al*., 1998).

According to the information available in the literature, *C. pseudodiphtheriticum* isolates are sensitive to β-lactams, aminoglycosides, rifampicin and tetracycline, whereas *C. accolens*, is sensitive to penicillin, cephalosporins, erythromycin, clindamycin, tetracycline and aminoglycosides (Funke *et al.,* 1997; Ustaçelebi, 1999; Brown, 2000). In this study, *C. pseudodiphtheriticum* strains were only 66% sensitive to penicillin, 33% to erythromycin and all isolates were resistant to clindamycin. *Corynebacterium accolens* isolates were 50% sensitive to penicillin, oxacillin, erythromycin and clindamycin.

In conclusion, emerging antimicrobial resistance in various species has created an additional need for accurate identification of coryneform organisms at the species level and continuous surveillance of their resistance patterns. In this study, the importance of the need to take coryneform bacteria into consideration especially as pathogens of opportunistic infections in ICU patients and the importance of assessing their antibiotic sensitivity is emphasized.

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