INTRODUCTION

Non-diphtheritic corynebacteria are aerobic and anaerobic, non-acid fast, pleomorphic, nonbranching, gram-positive rods that do not form spores. They are also called diphtheroids because of their morphologic resemblance with Corynebacterium diphtheriae. Human skin flora is very rich in corynemobacteria [1]. Hence when isolated from clinical specimens they are often neglected as skin contaminants. But recent reports of increased rate of isolation evidenced their potential as emerging nosocomial pathogens among immunocompromized patients (malignancy), patients on medical device, patients receiving broad spectrum antimicrobial therapy and after invasive procedures [2-4]. Some of the species like C. jeikeium and C. urealyticum can cause infections among immune-competent persons and are true pathogens. Emergence of antimicrobial resistance among various species of diphtheroids demands their species level identification [5]. But identification of diphtheroids to species level by routine as well as reference laboratories is confounded even after consulting all the available identification schemes [2]. Most of the research works concentrate on case reports and on particular species of diphtheroids [5].

AIM

The aim of the present study was to speciate non-diphtheritic corynebacteria isolated from wound specimens, to correlate their clinical significance and to determine their invitro antimicrobial susceptibilities to 9 antimicrobial agents.

MATERIALS AND METHODS

A prospective study on clinical samples from skin and soft tissue infections (pus, wound swab and tissue bits) submitted to the Clinical & Laboratory Standards Institute (CLSI) described broth microdilution method. MIC was interpreted according CLSI and British Society for Antimicrobial Chemotherapy (BSAC) guidelines.

Results: C. amycolatum was the predominant species (20%) followed by C. striatum (16%). Penicillin was least effective invitro followed by clindamycin and ciprofloxacin. Excellent activities were shown by vancomycin, linezolid and imipenem. Multidrug resistance was found in all the species.

Conclusion: Non-diphtheritic corynebacteria are potential nosocomial pathogens among acute/chronic complicated skin and soft tissue infection. Vancomycin or linezolid can be used empirically to treat such infections until the invitro susceptibility results are available.

Keywords: C. amycolatum, Diphtheroids, Multidrug resistance
of deionized water and using a sterile applicator stick several well isolated and similar colonies were smeared onto the surface. Development of red color within 5 minutes is considered positive for Beta-lactamase production.

STATISTICAL ANALYSIS
Statistical analysis was done using Epi Info™ 7.1.4 software program developed by Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia (USA). Simple frequencies were tabulated. Chi square test was done to determine the statistical significance. A p-value of < 0.05 was considered as statistically significant.

RESULTS
A total of 634 clinical samples from skin and soft tissue infections were studied. Twenty five single-patient isolates of genus Corynebacterium were included in the study (15 males and 10 females; mean age 47 years with range 22 to 76 years). Organisms were isolated from chronic non-healing ulcers, post slit skin graft raw areas, diabetic foot and post LSCS wound infection [Table/Fig-2]. All the patients were on broad spectrum antibiotics for prolonged period prior to sampling. The mean duration of hospital stay of patients was 14.5 days (range 7 to 30 days). Diabetes was found in 8 out of 25 cases.

Among the 25 isolates, 6 were obtained as pure growth and remaining were obtained as mixed growth, with diphtheroids as the predominant isolate [Table/Fig-3]. Direct smears stained with Gram’s stain showed inflammatory cells with gram positive bacilli in 28% of the samples. Twelve different species of corynebacterium were isolated [Table/Fig-4]. C. amycolatum was the predominant species (20%) followed by C. striatum (16%).

Overall antibiotic resistance pattern of the isolates showed high frequency of resistance to penicillin followed by clindamycin and ciprofloxacin. Excellent activities were shown by vancomycin, linezolid and imipenem [Table/Fig-4]. Beta-lactamase production was detected in 40% of isolates.

DISCUSSION
Corynebacterium spp can cause both acute and chronic wound infection [4,13]. When a non-diphtheritic corynebacteria is isolated from clinical specimen, detailed patient profile and repeat microbiological analysis should be done before reporting it as contaminant [2,10]. Antimicrobial susceptibility patterns of corynebacteria are not predictable and hence detection of antimicrobial susceptibility may be necessary in order to obtain the best therapeutic results.

In the present study all the diphtheroids were isolated from inpatients. Twenty four percent of samples grew diphtheroids in pure growth and 76% of isolates were obtained along with other bacteria. E coli (24%) was the commonest bacteria associated with diphtheroids followed by Klebsiella spp (12%), Pseudomonas aeruginosa (12%), Staphylococcus aureus (12%), Moraxella spp (8%), Enterococcus spp (4%) and Group C Streptococcus spp (4%). Initially cases were treated according to the susceptibility pattern of associated bacteria considering diphtheroids as possible skin commensals. After complete course of antibiotics, wound did not show any signs of healing and repeat culture obtained same diphtheroids. Customized therapeutic combinations were designed according to the sensitivity pattern of diphtheroids and associated microorganisms for each patient. Treatment with such regimen showed very good response. The isolation of Corynebacterium species from clinically apparent skin and soft tissue infection and healing of lesions after appropriate antibiotic therapy suggest the pathogenic role of these organisms in our patients.

Current CLSI guidelines recommend detection of MIC as standard method of determining antibiotic sensitivity. Though BSAC recommend MIC as the standard method, it also recommends use of disc diffusion testing for few antibiotics [12]. CLSI recommends detection of MIC using Mueller Hinton broth enriched with 5% lysed horse blood in microtiter plates as standard method [11]. Many laboratories fail to determine MIC because of its complex procedure and lack of technical expertise. Automated systems like Vitek 2/Phonix/API can determine sensitivity of diphtheroids [14]. But very few laboratories have this facility.

In the present study vancomycin (100%) was the most active drug against diphtheroids invitro followed by linezolid (96%) and imipenem (92%). Penicillin (0%) was least active drug followed by clindamycin (28%) and ciprofloxacin (32%). Similar findings were made by Soriano et al., and Camello et al., [5,15]. Though vancomycin is the most active drug, resistance to the drug has been reported in C. aquaticum and CDC group B1 [15]. An isolate of C. riegelii showed an MIC of 16 µg/ml against linezolid. Resistance break point to linezolid for diphtheroids is not defined and hence judicious use of this drug is essential in clinical practice. Imipenem resistance was noted in one isolate of CDC group G from a case of post slit skin graft wound infection and one isolate of C. urealyticum from diabetic ketoacidosis with carbuncle. Both the patients were previously treated with carbapenems for non-healing ulcers leading to selection of imipenem resistant corynebacteria. Multidrug resistance was found in all Corynebacterium spp. Nosocomial outbreak of clonal multidrug resistant Corynebacterium spp has been recently reported [16].
In this study, chronic non-healing ulcers, advanced age, diabetes, longer duration of hospital stay and prolonged antibiotic therapy were the risk factors for diphtheroids infection. Most of the patients were from male and female surgical wards (n=9), plastic surgery ward (n=6), gynecology post-operative ward (n=6), intensive care unit (ICU) (n=2) and medical wards (n=2). Higher occurrence of cases in particular wards and multi-drug resistance among the strains, suggest the probable nosocomial origin of these bacteria. Coyle et al., using plasmid profiling as an epidemiological tool showed diphtheroids spread in hospitals from person to person and airborne modes [2]. Environmental contamination of wards may be the common source for infection. Proper infection control and surveillance activities are needed to control such infections.

LIMITATION

Limitation of the present study was smaller sample size. Further study has to be done on isolates obtained from larger geographical area to know the exact prevalence of skin and soft tissue infections due to non-diphtheritic corynebacteria.

CONCLUSION

We conclude that non-diphtheritic corynebacteria are potential nosocomial pathogens among acute/chronic complicated skin and soft tissue infection. Wound infection with such organism causes delayed healing, raised treatment costs, and resource demanding wound management practices. We recommend vancomycin or linezolid be used empirically to treat complicated skin and soft tissue infections with non-diphtheritic corynebacteria and the ultimate therapeutic regimen against these organisms should be chosen according to the in vitro susceptibility results, the site of the infection and associated microorganisms.

REFERENCES


PARTICULARS OF CONTRIBUTORS: 1. Assistant Professor, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore, Karnataka, India. 2. Associate Professor, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore, Karnataka, India. 3. Post-Graduate Student, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore, Karnataka, India. 4. Post-Graduate Student, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore, Karnataka, India. 5. Post-Graduate Student, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore, Karnataka, India. 6. Post-Graduate Student, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR: Dr. Shoorashetty Manohar Rudresh, Assistant Professor, Department of Microbiology, ESIC MC & PGIMSR, Rajajinagar, Bangalore-560010, Karnataka, India. E-mail: rudreshsm@gmail.com

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