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CHARACTERISATION OF NONDIPHTHERIAL CORYNEBACTERIA ISOLATED FROM CLINICAL SAMPLES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PATTERN

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ABSTRACT

Introduction: Coryneform or the nondiphtherial *Corynebacterium* species remains a neglected group as contaminants. These organisms have been associated with invasive disease, particularly in immunocompromised patients. Species like *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, *Corynebacterium minutissimum* and *Corynebacterium urealyticum* are reported with increasing frequency. An alarming rate of antibiotic resistance is also documented among such organisms. **Aim and Objective:** This study was done to find out the various species of clinically relevant Coryneforms and to determine their antibiogram. **Materials and Methods:** A total of 857 clinical samples (Pus, wound swab, urine, blood, sputum and catheter tips) received in the Microbiology department during January 2013 to October 2013 were included in the study. They were subjected to Gram's staining and culture on blood agar and McConkey agar. The Gram-positive bacilli (GPB) isolated were subjected to speciation based on the panel of reactions described by Von Graevenitz and Funke. The antibiogram was determined by the disc diffusion method on 5% sheep blood agar. **Results:** Out of total 857 clinical samples, 42 (4.9%) samples showed pure growth of GPB. *C. jeikeium* was the predominant species isolated. Species like *C. jeikeium* and *C. urealyticum* were highly resistant to commonly used antibiotics. **Conclusion:** Coryneforms can no longer be ignored as contaminants. Determining the antibiogram is necessary as species like *C. jeikeium* and *C. urealyticum* are highly resistant to commonly used antibiotics. In our study all these organisms are sensitive to vancomycin, linezolid and tigecycline. Hence these drugs can be considered in the empirical treatment against serious infections caused by these organisms.

Key words: *Corynebacterium* jeikeium, Coryneforms, Tigecycline, Vancomycin

INTRODUCTION

Non-diphtherial corynebacteria, which are also referred to as diphtheroids, are a widely diverse collection of bacteria.[1] These catalase-positive, non-sporing Gram-positive bacilli (GPB) were always dismissed as contaminants when recovered from patients. These coryneform bacteria are increasingly recognized as causing opportunistic disease under specific circumstances, such as in patients who are immunocompromised, have prosthetic devices or have been in hospitals for long duration.[2] These organisms have been implicated in multiple infections like catheter-associated blood stream infections, endocarditis, prosthetic valve infections, meningitis, neurosurgical shunt infection, brain abscess, peritonitis, osteomyelitis, septic arthritis, urinary tract infections, empyema and pneumonia.[1] Species like *Corynebacterium amycolatum*, *Corynebacterium jeikeium*, *Corynebacterium minutissimum*, and *Corynebacterium urealyticum* are being reported with increasing frequency in recent years.[3] They are prominent contaminants of clinical materials; hence it is difficult to correctly decide if recovery of such bacteria implies contamination or has clinical relevance.[2] Therefore, it is recommended that these organisms should be identified to the genus and species level whenever they grow in pure culture from clinical specimens and/or when they represent the predominant organisms in normally sterile samples.[4] It is worthwhile to identify coryneform bacteria to the species level in order to detect unsuspected species and to ascribe potential pathogenicity to species so far thought to be nonpathogenic. Antimicrobial susceptibility patterns of coryneform bacteria have not been studied systematically.[4] Most recent studies show an alarming rate of antibiotic resistance among these organisms.[5] Resistance to β-lactams, clindamycin, erythromycin, azithromycin, ciprofloxacin, and gentamicin is quite frequent, with vancomycin,

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doxycycline, fusidic acid and prystinamycin being the agents that are most active *in vitro*.[6]

Aims and objectives

This study was done

1. To find the different species of clinically relevant coryneform bacteria isolated from various clinical materials and
2. To determine their antibiotic susceptibility pattern.

MATERIALS AND METHODS

This prospective study was done during the period of January 2013 to October 2013. A total of 857 clinical samples (Pus, wound swab, urine, blood, sputum and catheter tips) received in the Microbiology department during this period were included in the study. Direct gram staining was done for all samples except blood. The samples were inoculated on sheep blood agar and McConkey agar plates and incubated for 48 h at 37°C. All the samples which showed pure growth of GPB were subjected to further study. The true clinical significance of these GPB were based on the following criteria: (i) GPB are present in direct Gram-stain along with pus cells (ii) when isolated from normally sterile body sites, e.g. blood culture, (iii) from adequately collected clinical material where coryneforms are the predominant organism and (iv) when repeatedly isolated.[7]

Speciation was done based on the panel of reactions described by von Graevenitz and Funke,[1] which included a motility, catalase test, nitrate reduction, test for fermentation or oxidation, acid production from glucos, maltose, sucrose, mannitol and xylose, urea hydrolysis, esculin hydrolysis, Christie, Atkins, Munch-Peterson reaction with a beta hemolysin producing strain of *Staphylococcus aureus* and a test for lipophilism.

The antibiogram was determined by the disc diffusion method on 5% sheep blood agar. About 10^8 CFU/ml of the organisms were inoculated on the plates[8] and the following antibiotic discs were used: Ampicillin (10 µg), amoxycillin-clavulanic acid (20/10 µg), cefaperazone-sulbactum (75/10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), clindamycin (2 µg), erythromycin (15 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), gentamicin (10 µg), imipenem (10 µg), linezolid (30 µg), oxacillin (1 µg), penicillin (10 units), piperacillin-tazobactum (100/10 µg), tetracycline (30 µg), vancomycin (30 µg) and tigecycline (15 µg).[1] The plates were incubated at 37°C for 24 h. ATCC *S. aureus* 25923 was used as control strain.

Evaluation criteria

Due to lack of established Clinical and Laboratory Standards Institute (CLSI) guidelines for this group of organisms, the results were interpreted based on a combination of CLSI guidelines applicable for *S. aureus* and the British Society for Antimicrobial Chemotherapy guidelines for testing ciprofloxacin, penicillin and vancomycin.[5,8,9]

RESULTS

Out of total 857 clinical samples, 42 samples showed pure growth of GPB constituting about 4.9%.

DISCUSSION

Out of the 42 isolates studied, 19 (45.24%) were obtained from pus sample and nine (21.43%) from wound swab. This correlates with the study conducted by BS Reddy[1] in which 32.4% of GPB were isolated from pus sample. This shows that these organisms normally present as skin commensals can cause infection under specific circumstances. Seven isolates were isolated from urine sample (16.66%), four from sputum (9.52%), two from catheter tip (4.76%) and one isolate from blood (2.38%) [Table 1].

Among the non diphtherial *Corynebacterium*, *C. jeikeium* (45.24%) was the predominant species isolated in our study followed by *Corynebacterium* ulcerans (30.95%). *C. jeikeium* was commonly isolated from pus, wound swab and urine samples. The other species isolated were *Corynebacterium pseudodiphtheriticum* (9.52%), *C. urealyticum* (9.52%) and *C. durum* (4.76%). *C. pseudodiphtheriticum* was isolated only from sputum samples and *C. urealyticum* was isolated from urine

Table 1: Distribution of isolates in various clinical samples

Sample	Number of isolates (*n*=42)	Percentage
Urine	7	16.66
Pus	19	45.24
Blood	1	2.38
Sputum	4	9.52
Wound swab	9	21.43
Catheter tip	2	4.76

Majority of isolates were obtained from pus and wound swab followed by urine and sputum as shown in Table 1

samples only. This finding also correlates with the study conducted by Reddy [Figure 1].[1]

As far as the various species are concerned, differences have been observed in identification of *Corynebacterium* species recovered from the clinical samples. No uniformity has been observed in this regard.[1] Most clinical laboratories encounter difficulties with identification of coryneform isolates. One of the reasons for the difficulty with identifying coryneform species is the lack of deoxyribonucleic acid relatedness studies to confirm homogeneity within recognised taxa.[10]

Most of the isolates showed high frequency of resistance to multiple antibiotics. Increased resistance was shown to penicillin, ampicillin, gentamycin and erythromycin. In our study all the isolates were sensitive to vancomycin, linezolid and tigecycline.

β-lactam antibiotics showed least activity against the coryneforms with a resistance of 78.57% against penicillin, 73.81% to ampicillin and 69.05% to ceftriaxone. Oxacillin was also found to be least effective with a resistance rate of 71.43%. Combination of β-lactamase inhibitors was also not found to be much active against these organisms which showed a resistance rate around 65%. These findings were similar to the earlier studies which also prove coryneforms are highly resistant to β-lactam antibiotics with resistance of 65%.[1,4,5] Our finding is in contrast to the study conducted by Funke[9] which shows penicillin to be effective against coryneform bacteria [Table 2].

A high rate of resistance (76.19%) was also observed against gentamycin. The isolates also showed increased resistance to erythromycin (76.19%) and clindamycin

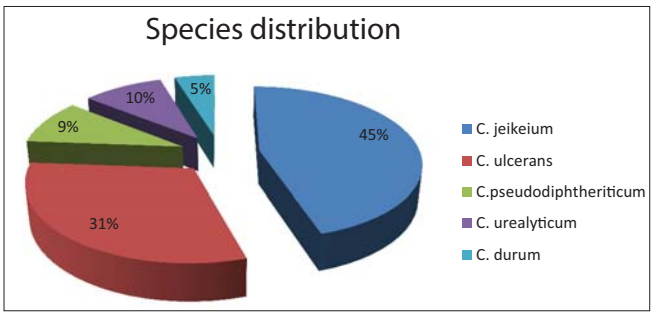

Figure 1: Percentage of various species of nondiphtherial *Corynebacterium*. (*Corynebacterium jeikeium* was the predominant species isolated in our study followed by *Corynebacterium ulcerans* as shown in Figure 1)

(71.43%). This is similar to the study conducted by Soriano[5] which demonstrated a higher incidence of erythromycin resistance among the coryneforms. Hence, erythromycin once considered the drug of choice for treatment of infections caused by these organisms can no longer be recommended.[5]

Ciprofloxacin and tetracycline shows moderate activity against coryneforms with a sensitivity of 54.76% and 57.14% respectively. Chloramphenicol was found to have good activity with sensitivity rates of 71.43%. Imipenem was also found to be active against coryneforms with a sensitivity rate of 78.57%.

In our study, vancomycin, linezolid and tigecycline were the most sensitive antibiotics which were active against all the isolates with 100% sensitivity. This also correlates with the previous studies which showed vancomycin to be the most effective drug against the coryneforms.[11] This suggests that glycopeptide antibiotics can be used in the empirical treatment of infections caused by coryneform bacteria. Tigecycline, the newer antibiotic can also be considered for treatment as a substitute to glycopeptide antibiotics.[1,5]

Corynebacterium jeikeium was the predominant species which showed high level of resistance to multiple antibiotics. They were resistant to β-lactams,

Table 2: Antibiotic susceptibility pattern of the isolates

Antibiotics	Number of resistant isolates (*n*=42)	Percentage
Ampicillin	31	73.81
Amoxycillin-clavulanic acid	27	64.28
Cefaperazone-sulbactum	28	66.60
Ceftriaxone	29	69.05
Chloramphenicol	12	28.57
Clindamycin	30	71.43
Erythromycin	32	76.19
Ciprofloxacin	20	47.62
Nitrofurantoin	25	59.52
Gentamycin	32	76.19
Piperacillin-tazobactum	26	61.90
Tetracycline	18	42.86
Oxacillin	30	71.43
Penicillin	33	78.57
Imipenem	9	21.43
Linezolid	0	0
Vancomycin	0	0
Tigecycline	0	0

A high level of resistance was seen against penicillin, gentamycin, erythromycin and ampicillin

aminoglycosides and macrolides. Quinolones were also not effective. Moderate level of activity was exhibited by chloramphenicol, tetracycline and imipenem. All the isolates of *C. jeikeium* were susceptible to vancomycin, linezolid and tigecycline. This finding correlates with the previous studies,[1,4,8] which also demonstrates *C. jeikeium* to be the most resistant species of coryneforms.

Next to *C. jeikeium*, *C. urealyticum* was the highly resistant species. It was resistant to β-lactams, aminoglycosides and macrolides. Nitrofurantoin was seen to exhibit moderate level of activity against *C. urealyticum* with a sensitivity rate of 50%. They were also found to be sensitive to ciprofloxacin with 75% sensitivity rate. Vancomycin, linezolid and tigecycline showed 100% sensitivity against these organisms.

CONCLUSION

Corynebacterium species, other than *Corynebacterium diphtheriae* are being reported with increasing frequency as potential pathogens especially in hospitalized patients due to their state of immunosuppression. Therefore, when isolated in pure form they can no longer be ignored as contaminants. Drug resistance among these bacteria is well documented and determining the antibiogram is necessary as species like *C. jeikeium* and *C. urealyticum* are highly resistant to commonly used antibiotics like β-lactams, aminoglycosides and macrolides. Our study shows that all these organisms are highly sensitive to vancomycin, linezolid and tigecycline. Hence these drugs can be considered in the empirical treatment against serious infections caused by these organisms.

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