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Coryneforms causing surgical site infections- characterization and antimicrobial sensitivity pattern

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ABSTRACT

Coryneforms have been recognized as pathogenic organism with increasing frequency in recent years and can no longer be discarded as laboratory contaminants. Emerging antimicrobial resistance in various species has created an additional need for their identification at the species level and continuous surveillance of their antimicrobial resistance pattern. This study was conducted to identify the role and resistance pattern of *coryneforms* in the surgical site infections. *C. jeikeium* was found to be the commonest species among the 54 isolates of *coryneforms*. Furthermore alarming resistance profile was observed among all the species. One of the important highlight of the study was the unexpected synergism noted between erythromycin & vancomycin and pristinomycin & vancomycin. On the basis of our results we would like to conclude that the role of *coryneforms* in surgical site infections really needs to be looked into and the synergistic activity among the erythromycin/ pristinomycin and vancomycin will prove to be helpful in delaying the development of resistance against vancomycin which is the need of time.

Keywords: Coryneforms, surgical site infection, antimicrobial resistance.

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INTRODUCTION

Surgical site infection (SSI) is one of the commonest healthcare associated infections worldwide. By definition SSI is an infection which occurs within 30 days of surgery (or up to 1 yr after surgery in patients receiving implants) affecting either the incision or the deeper tissue at the operation site.[1] Despite improvements in prevention, SSIs remain a significant clinical problem as they are associated with substantial mortality and morbidity and impose severe demands on healthcare resources. Several pathogens have been associated with infections at surgical sites like *Staphylococcus aureus, CONS, Enterococcus spp., Pseudomonas aeruginosa, E.coli, Klebsiella spp., Acinetobacter spp., Citrobacter spp.* and various non fermenter gram negative bacilli.[2,3] However the role of *coryneforms* in these infections has been little studied. After decades of confusion about their clinical significance, *coryneforms* have emerged as important pathogens.[4] With the increase in survival rate of severely immune compromised patients as well as increased use of medical devices in such patients control of opportunistic infections due to *coryneform* bacteria is of immense importance.[5] There are very few published studies regarding the role of *coryneforms* in SSIs and none of them have been from Indian subcontinent therefore we decided to evaluate the role of *coryneform* bacteria in SSIs.

MATERIAL AND METHOD

Place and Time of Study:

Jawaharlal Nehru Medical College (JNMCH), Aligarh from September 2008 to May 2010. All the patients who were admitted in different surgical units of JNMCH with SSIs were included in the study. A sterile syringe / sterile wound swab was used to collect the pus at the incision site and cultured on 5% sheep blood agar and Mac Conkey agar at 37°C for 24-48 hours. A total of 54 consecutive isolates of *coryneforms* were obtained from various clinical samples. The recovered strains were routinely submitted for culture to the microbiology laboratory so no written consent for inclusion in the study was required from the patients.

All the *coryneforms* thus obtained were further characterized on the basis of gram staining, morphology, colony character and following tests: catalase and nitrate reaction, motility, lipid requirement (pin point colonies were considered as lipophilic while pin head size colonies were considered as non-lipophilic), urease test, esculin and gelatin hydrolysis, CAMP reaction, sugar fermentation test (glucose, maltose, sucrose, xylose, mannitol and lactose).

Antimicrobial susceptibility testing was determined using 5% sheep blood agar in Muller Hinton agar by Kirby-Bauer disk diffusion method using an inoculum of approximately 105 CFU/ml.

Antibiotics tested included-Gentamicin, cotrimoxazole, erythromycin, amikacin, ofloxacin, gatifloxacin, vancomycin, oxacillin, pristinamycin. The susceptibility criteria of the CSLI for *Staphylococcus spp*. were used for all antibiotics. Given the multidrug character of *coryneforms* we looked for synergy among different groups of drugs vis-à-vis aminoglycosides and erythromycin, fluoroquinolones and oxacillin, vancomycin and macrolides and vancomycin and pristinamycin by disc diffusion method.

Method of synergy testing:

Synergy between vancomycin and pristinamycin/ erythromycin was detected by placing a disc of vancomycin and a disc of pristinamycin/ erythromycin, 15mm and 20mm apart (centre to centre) on an inoculated agar plate. A clear potentiation of the edge of the vancomycin inhibition zone towards the disc containing erythromycin/ pristinamycin was interpreted as synergy. To the best of our knowledge in-vitro synergy between these two drugs has been observed for the first time in this study.

RESULTS AND DISCUSSION:

Despite improvement in prophylaxis and infection control measures, SSI remains an important cause of nosocomial morbidity and mortality. This in turn imposes heavy demands on health care resource due to prolonged hospitalization. *Non diptherial coryneforms*, originally thought to be mainly contaminants, have been recognized recently as pathogenic especially in immunocompromised hosts or in patients with indwelling catheters.[6]

During the course of 21 months, 54 clinically significant coryneforms were isolated from SSIs. Majority of these isolates were *C.jeikeium*, isolated from 26 cases (48.1%), followed by *C. striatum* and *C. amycolatum* as seen in Table I. One isolate each of *A. hemolyticum* and *A. pyogenes* was also identified.

Table I:- Coryne bacterium spp in surgical site infection(n=54)

Corynebacterium jeikeium	26(48.1%)
Corynebacterium striatum	18(33.3%)
Corynebacterium amycolatum	10(18.5%)
Arcanobacterium hemolyticum	1(0.02%)
Arcanobacterium pyogenes	1(0.02%)

C. jeikeium, the predominantly isolated organism in our study, causes a variety of infections including septicemia, meningitis, peritonitis, foreign body infection with i.v. devices, osteomylitis, pneumonitis and endocarditis. These infections are often nosocomial, occurring in patients with compromised host defenses, exposure to broad spectrum antimicrobials or

prolonged hospitalization or a combination of any of these factors.[7] A new trend, however, has been an increasing recognition of *C. jeikeium* infections in immunocompetent host.[8] *C. jeikeium* has been reported to cause sepsis primarily in patients with neoplastic disease with associated risk factors such as prolonged hospitalization, neutropenia, treatment with multiple antibiotics and disruption on the skin surface.

Majority of *coryneforms* were isolated from SSIs in orthopaedics surgeries (50%) followed by general surgeries (35.4%) and obstetrics and gynaecology(14.8%)Table II.

Table II:- Corynebacterium spp. isolates from different surgical units(n=54)

Surgical department	Numbers of isolates
Orthopaedics	27(50%)
General surgery	19(35.2%)
Obstetrics and Gynaecology	08(14.8%)
Ophthalmology	-
E.N.T	-

Antimicrobial resistance profile of different species is given in Table III. In our study the degree of resistance to all the antimicrobials used was alarming except vancomycin. All the isolates were uniformly resistant to co-trimoxazole and amikacin. Amongst all the coryneforms *C. striatum* had a slightly better antimicrobial susceptibility pattern followed by *C. jeikeium*. It has been observed during the study that coryneforms were slightly less resistant to oxacillin and pristinomycin than others drugs tested. Antimicrobial susceptibility studies revealed that a preponderant proportion of the reported *C. jeikeium* isolates are substantially multiresistant against clinically relevant antibiotics and that only glycopeptides, such as vancomycin and teicoplanin, remain universally active against this species.[4,9,10]

Discussing separately, all the isolates of *C jeikeium* were resistant to fluoroquinilones, aminoglycosides, macrolides and cotrimoxazole; 53.8 % were resistant to oxacillin and 57.6% were resistant to pristinamycin while all of them were sensitive to vancomycin. This emergence of multiresistant phenotypes largely limits the therapeutic options and thus has tremendous consequences for successful treatment of *C. jeikeium* infections, especially in immunocompromised patients. Up to now, the molecular basis for multiresistance of *C. jeikeium* against antimicrobial agents remained unexplained. A few studies, however, have investigated the presence of plasmids in *C. jeikeium*, indicating that the multiresistant phenotype is encoded by the bacterial chromosome rather than associated with extrachromosomal DNA.[11] Accordingly, it was concluded that the multiresistance of *C. jeikeium* is a consequence of the accumulation of specific genetic events and/or may involve a set of nonspecific mechanisms,

such as increased antibiotic efflux or changes in the permeability of the corynebacterial cell wall.[12]

Table III:- Antibiotic resistance profile of *Corynebacterium species*

Coryneform sp	G	E	Co	Ox	A	O	Ga	P	Va
C.jeikeium	100	100	100	53.8	100	100	100	57.6	0
C.striatum	100	83.3	100	77.7	100	100	94.4	88.8	0
C.amycolatum	100	100	100	100	100	100	100	100	0
A.hemolyticum	100	100	100	100	100	100	100	100	0
A.pyogenes	0	0	100	0	100	0	100	0	0

G-Gentamycin, E-Erythromycin, Co-Co-trimoxazole, Ox-Oxacillin, A-Amikacin, O-Ofloxacin, Ga-Gatifloxacin, P-Pristinamycin, Va-Vancomycin

Unlike C. jeikeium, C. striatum has been found as a part of normal flora of the human nasal passages and on human skin. It has been increasingly reported as an emerging pathogen in compromised individuals. It has been recovered in patients with osteomyelitis, septic arthritis and synovitis, pulmonary abscesses, breast abscesses, meningitis, CSF shunt infection and CAPD associated peritonitis. Like *C. jeikeium* it is also a multiresistant coryneform.[13] All the *C. striatum* isolates of our study were susceptible to vancomycin. But majority were resistant to erythromycin (83.3%), oxacillin(77.7%), gatifloxacin (94.4%) and pristinamycin (88.8%). Whereas all of them were resistant to gentamicin, co-trimoxazole, Amikacin and ofloxacin.

The third most abundant organism, *C. amycolatum,* has been isolated from significant number of human infections. It has been isolated from catheter related infection, surgical wound infection, mastitis, native valve and nosocomial endocarditis and septic arthritis. Like the above two *coryneforms* this organism is also multiresistant.[14] In our study *C. amycolatum* isolates were resistant to all the antibiotics used except for vancomycin.

Archanobacterium species have been isolated from variety of clinical infections in humans including pharyngitis, chronic skin ulcers, soft tissue infections, deep tissue abscesses, joint space infections, sinusitis, orbital cellulitis, pneumonia, osteomyelitis, endocarditis and meningitis. Usually they are sensitive to antimicrobials like penicillin, cephalosporins, macrolides and vancomycin but resistant isolates have also been reported.[15,16,] Only two of our isolates were identified as *Archanobacterium species* , one was *A hemolyticum* and the other was *A pyogenes*. *A. hemolyticum* was multiresistant and showed resistance to all the drugs tested except vancomycin. However, *A pyogenes* was found to be sensitive to vancomycin, gentamycin, erythromycin, oxacillin and pristinamycin.

Few studies have documented sensitivity to oxacillin, so it is unclear whether *coryneforms* have developed resistance to oxacillin over the period of time due to alteration of penicillin binding

protein or there is intrinsic resistance to oxacillin in these species.

Different combinations of drugs were studied for synergy. Unexpected synergism was noted between pristinamycin and erythromycin with vancomycin in number of *corynebacterium* isolates even when a number of these isolates were either resistant to one of them or both. Synergy was noticed between vancomycin and pristinamycin in 55.3%(30/54) isolates and between vancomycin and erythromycin in 3.0%(2/54) of isolates even when many of the isolates were resistant to pristinamycin and erythromycin. In case of pristinamycin better synergy was observed when the disc was placed at a distance of 15mm whereas erythromycin showed better results when the discs were placed 20mm apart (Figure 1 and 2).

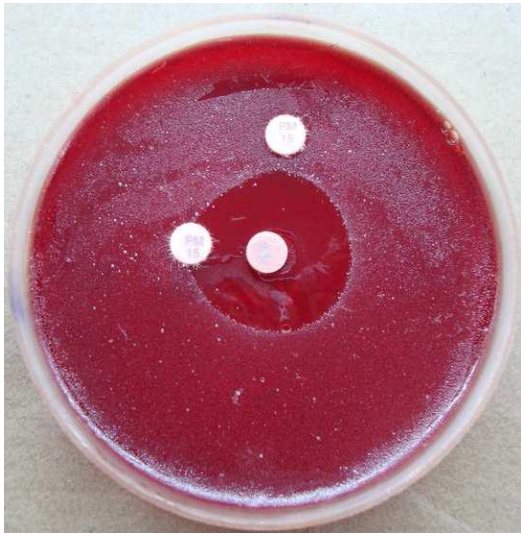

Figure 1. Showing synergy between Pristinomycin and Vancomycin.

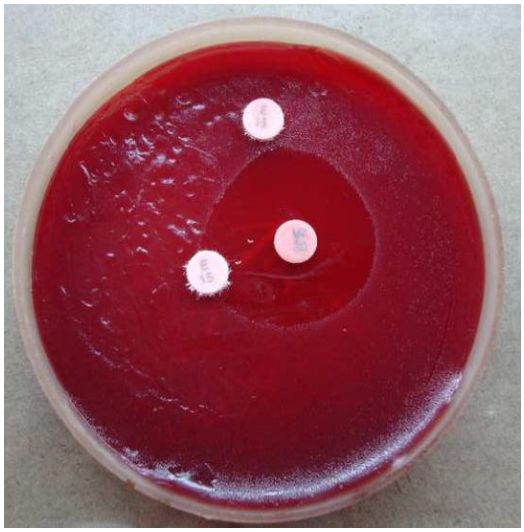

Figure 2. Showing synergy between Vancomycin and Erythromycin.

On the basis of these findings, we recommend that rather than using vancomycin alone it would be advisable to use pristinamycin or erythromycin along with vancomycin to prevent

development of resistance to vancomycin as faster cure could be achieved. However, more work needs to be done on this.

CONCLUSION:

Corynebacterium is an emerging pathogenic organism and its role in the surgical site infections really needs to be looked into as and it has been increasingly recognized as a multidrug resistant. Furthermore, the synergistic activity between erythromycin, pristinomycin and vancomycin will prove to be helpful in the delaying the development of resistance of higher antibiotics which is the need of time.

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