



Original

An Observational Study of Community-Acquired MRSA from Head and Neck Space Infections in Northern India

Binod Kumar Pati*³, S Krishna Prakash¹, A K Agarwal², B Uppal¹ and A Gulati²

¹Department of Microbiology, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, 110002, India

²Department of Otorhinolaryngology, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, 110002, India

³Department of Microbiology³ Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, 226014 U.P. India

ARTICLE INFO

Received 20 July 2015
Received in revised form 07 Aug. 2015
Accepted 14 Aug. 2015

Keywords:

DNIs,
MRSA,
Otorhinolaryngology,
North India.

Corresponding author: Department of Microbiology, Sanjay Gandhi Post graduate institute of medical sciences, Rae bareli Road, Lucknow, India, PIN - 226014.

E-mail address: drbkpati@gmail.com

ABSTRACT

Background: Deep neck infections (DNIs) continues to be encountered in daily clinical practice even in the settings of widespread antibiotic use. We investigated the microbiology of deep neck infections and antibiogram profiles of *Staphylococcus aureus* isolates.

Methods: Isolation and characterization of *Staphylococcus aureus* strains were performed from pus samples aspirated from clinically suspected patients of DNIs attending the Otorhinolaryngology department of a tertiary care health setting in northern India.

Results: *Staphylococcus aureus* was the most commonly recovered agent, out of which 11.5% were methicillin-resistant (MRSA).

Conclusion: Community-acquired MRSA (CA-MRSA) from deep neck infections is an issue of grave concern and emphasizes the need for culture-directed clinical decisions and appropriate choice of antibiotics.

Introduction

Deep neck infection is defined as a suppurative infectious process of the neck that often starts as a soft tissue cellulitis and eventually leads to abscess¹. It could be lymphadenitis, cellulitis, necrotic node or abscess in nature². *Staphylococcus aureus* (*S. aureus*) is one of the commonest gram positive aerobic bacteria in DNIs besides the Streptococci. Antibiotic resistance is a matter of grave concern. While it is well-known that MRSA infections have become

endemic in hospitals, and usually afflict patients with established risk factors, more recently MRSA infections have also been described in patients without established risk factors and these infections involving the community are referred to as community-acquired MRSA (CA-MRSA) infections³. A significant morbidity and mortality is attributed to it. In one study⁴ a mortality of 5.9% and 6.2% was demonstrated in adults and children respectively. Monitoring the



spread of MRSA by epidemiological surveillance, especially in DNIs should be considered seriously to initiate aggressive treatment strategies for efficient management^{3,5}.

Whilst the microbiology of DNI varies depending on certain underlying factors and geographic locations, the aetiology of DNIs generally is polymicrobial⁶. While aerobic bacteria are commonly isolated, the presence of anaerobes may be underestimated because of the difficulty in culturing them⁷. We investigated the antimicrobial susceptibility pattern of *S. aureus* isolated from DNIs from a tertiary care clinical setting in northern India.

Materials and Methods

After taking informed consent, pus specimens were collected from 56 consecutive clinically suspected cases of DNIs in a tertiary care hospital. Patients with 48 hours prior history of antibiotic treatment and cases of tuberculosis were excluded. Samples were promptly transported to Microbiology department and processed within 30 minutes of collection. Both aerobic and anaerobic cultures were attempted. Plates incubated aerobically were read the next day and cultures reporting no growth, were incubated further with subcultures made from BHI broth. Anaerobic cultures were examined after 48 hours and in case of no growth on plates, subcultures were made from cooked meat broth and subsequently incubated anaerobically for another 48 hours. Bacterial isolates from cultures were identified and characterized following standard techniques. Grouping of the β -haemolytic streptococci isolates were performed by latex agglutination kit of Plasmatec Ltd (UK).

All the isolates were subjected to antimicrobial susceptibility testing to a wide battery of antibiotics by disc diffusion

method using modified Stokes' technique⁸. Isolates of *E. coli* and *K. pneumoniae* resistant to one or more of the third generation cephalosporins were subjected to detection of extended-spectrum β -lactamase (ESBL) using three established methods, namely double-disk synergy, phenotypic confirmatory combined disc test and ESBL E-test⁹. All the *S. aureus* isolates were tested for methicillin resistance by standard oxacillin screening agar test and cefoxitin disc method^{10,11}.

The *S. aureus* isolates were phage typed employing the conventional set of phages described by Blair and Williams¹². The MRSA isolates were further biotyped based on Tween-80 hydrolysis, urease production, pigmentation and gentamicin susceptibility and phenotyped based on their antibiogram^{13,14}.

Results

A total of 53 bacteria were isolated. *S. aureus* was the most common isolate accounting for 49% of all the bacterial isolates. 11.5% of these isolates were resistant to methicillin. While methicillin-susceptible isolates were recovered from all the six neck spaces investigated, the MRSA isolates primarily confined to the sites of infection namely, the submandibular, submental and peritonsillar spaces. Of the 12 isolates of β -hemolytic streptococci, eight were isolated in pure growth while four occurred in conjunction with *S. aureus*. Among them 09 belonged to Group A, two to Group F and one belonged to Group G streptococcus. Other aerobic pathogens isolated were *Enterococcus faecalis* (1), *Gemella morbillorum* (1), *Streptococcus mitior oralis* (1), *Escherichia coli* (2), *Klebsiella pneumoniae* (2), *Pseudomonas aeruginosa* (1). Anaerobic spectrum constituted 13.2% of the bacterial aetiology and of the seven anaerobes recovered, *Peptostreptococcus* species were

predominant (85.7%) followed by *Bacteroides* species.

D-test performed with a single clinical isolate of MRSA tested positive confirming the presence of erm-mediated resistance to clindamycin. MIC testing by E-test revealed that, all the methicillin-susceptible *S. aureus* (MSSA) isolates were uniformly sensitive to vancomycin, teicoplanin, mupirocin and fusidic acid. All the three MRSA isolates were sensitive to vancomycin, teicoplanin and mupirocin. Whilst two isolates were sensitive to fusidic acid, one showed high level resistance at >256 µg/mL. The comparative antibacterial susceptibility profile of MRSA and MSSA is listed in table 1.

The 26 *S. aureus* isolates subjected to phage typing by the conventional (basic) set of phages, the three MRSA isolates were found to be non-typeable, whereas 14 (60.9%) MSSA isolates were typeable. An overall typeability of 53.9% was observed for the staphylococci isolates. Maximum typeability was observed in the mixed group of phages (39.1%) followed by Group I (13.1%). None of the isolates were typeable by phages belonging to group II and the non-allocated group. The various phage patterns seen within each group for the typeable isolates of MSSA are shown in Table 2.

Biotyping of MRSA isolates showed that all the 3 MRSA isolates belonged to biotype A. Based on the antibiogram pattern, the 3 different isolates of MRSA showed 3 distinct mnemonic codes, the resistant phenotypes namely Bc (33.3%), Da (33.3%) and Ag (33.3%).

All the 12 isolates of β -hemolytic streptococci isolated irrespective of their serogroups and both the α -hemolytic isolates were susceptible to all the antibiotics tested. All the *E. coli* and *K. pneumoniae* showed resistance to all third-generation cephalosporins tested and were also tested

positive for the presence of extended-spectrum β -lactamases (ESBL) by all the methods employed herein. Both the *E. coli* and *Klebsiella* isolates, that were ESBL positive were subjected to MIC testing and the results showed that all the isolates were sensitive to Imipenem and Meropenem.

Discussion

As with previous reports, Gram positive cocci (GPC) were the most commonly recovered bacteria followed by GNB^{3,5,15,16} in our study. Recovery of ESBL producing *E. coli* and *K. pneumoniae* from aspirated pus underscores the magnitude of resistance demonstrated by these isolates and the significance of prescribing ESBL inhibitor group of antibiotics to the afflicted population. Evidence that MRSA is a growing clinical problem has been very well documented in the literature with increasing frequency over the past decade and more recently the increasing incidence of CA-MRSA infections has been the source of grave concern. All of the MRSA cases reported in our study were community-acquired. The increasing incidence of CA-MRSA infections has important clinical implications while approaching a patient presenting with head and neck space infections since empirical treatment is advised before culture results are available and now it is clear that careful consideration should be given to cover MRSA^{3,5}. Thus, it becomes the responsibility of physicians to be aware of the prevalence of CA-MRSA in communities and its antimicrobial susceptibility patterns and prescribe empirical treatment accordingly in cases of deep neck infections. Hence, we suggest that CA-MRSA be considered a potential challenge in head and neck space infections and a high index of suspicion and aggressive treatment is believed to be the prime requisite to prevent untoward complications.

An essential issue surrounding the use of clindamycin for the treatment of MRSA infections is the probable risk of treatment failure if the infection is by erythromycin-resistant *S. aureus* with the potential for selecting for clindamycin resistance⁵. Thus, determination of presence of erythromycin-inducible clindamycin resistance by D-test becomes arterial and one of the MRSA isolates tested positive for D-test emphasizes the need for constant monitoring of the clinical isolates.

Conclusion

This study supports the notion that CA-MRSA infections are an important part of the differential diagnosis when approaching a patient with head and neck space infection. It is essential to consider the rising incidence of MRSA when choosing the antibiotic while waiting for culture and susceptibility results. These days many alternative antibacterial strategies are also under trial leading to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-based products like *Nigella sativa*¹⁷. Cultures are therefore very critical in the diagnosis and management of head and neck space infections.

Acknowledgment

All the laboratory staff of Antibiotic susceptibility testing laboratory, Department of Microbiology, Maulana Azad medical college, New delhi, India

Conflict of interest

No financial support was received for this work. There is no conflict of interest to be declared by the authors.

References

1. Lin RH, Huang CC, Tsou YA, Lin CD, Tsai MH, Chen TH, Chen CM, Shiao YT. Correlation between imaging characteristics and

- monitoring in patients with deep neck infections: A retrospective review of one hundred sixty one cases. *Surgical Infection* 2014; 15(6):794-9
2. Huang TT, Tseng FY, Yeh TH, Hsu CJ, Chen YS. Factors affecting the bacteriology of deep neck infection: a retrospective study of 128 patients. *Acta Otolaryngol.* 2006; 126:396–401.
3. Ossowski K, Chun RH, Suskind D, Baroody FM (2006). Increased isolation of Methicillin-resistant *Staphylococcus aureus* in pediatric head and neck abscesses. *Arch Otolaryngol Head Neck Surg* 132:1176–81.
4. Santos Gorjon P, Blanco Perez P, Morales Martin AC, del Pozo de Dios JC, Estevez Alonso S, Calle de la Cabanillas M. Deep neck infections: Review of 286 cases. *Acta Otorrinolaryngologica Espanola*, 2012;63 (1):31-41
5. Naidu SI, Donepudi SK, Stocks RM, Buckingham SC, Thompson JW (2005). Methicillin-resistant *Staphylococcus aureus* as a pathogen in deep neck abscesses: A pediatric case series. *Intl J Pediatr Otorhinolaryngol* 69:1367–71.
6. Vieira F, Allen SM, Stocks RMS, Thompson JW. Deep neck infection *Otolaryngologic Clinics of North America*, 2008;41:459-483
7. Courtney MJ, Miteff A, Mahadevan M. *Management of pediatric lateral neck infections: Does the adage "... never let the sun go down on undrained pus ..." hold true?* *Int J Pediatr Otorhinolaryngol* 2007;71:95-100.
8. Gosden PE, Andrews JM, Bowker KE, Holt HA, MacGowan AP, Reeves DS, Sunderland J, Wise R (1998). Comparison of the modified Stokes' method of susceptibility testing with results obtained using MIC methods and British Society of Antimicrobial Chemotherapy breakpoints. *J Antimicrob Chemother* 42:161–9.
9. Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing. Twenty second informational supplement update. CLSI document M100-S19 U Clinical and Laboratory Standards Institute, Wayne, PA
10. Jain A, Agarwal A, Verma RK (2008). Cefoxitin disc diffusion test for detection of

- methicillin-resistant staphylococci. *J Med Microbiol* 57:957–61.
11. Anand KB, Agrawal P, Kumar S, Kapila K (2009). Comparison of cefoxitin disc diffusion test, oxacillin screen agar and PCR for *mecA* gene for detection of MRSA. *Ind J Med Microbiol* 27:27–9.
 12. Gustafson JE, O'Brien FG, Coombs GW, Malkowski MJ, Grubb WB, Pfeltz RF, Wilkinson BJ (2003). Alterations in phage-typing patterns in vancomycin-intermediate *Staphylococcus aureus*. *J Med Microbiol* 52:711–4.
 13. Coia JE, Thomson-Carter F, Baird D, Platt DJ (1990). Characterisation of methicillin-resistant *Staphylococcus aureus* by biotyping, immunoblotting and restriction enzyme fragmentation patterns. *J Med Microbiol* 31:125–32.
 14. Gupta N, Prakash SK, Malik VK, Mehndiratta PL, Mathur MD, (1999). Community acquired methicillin resistant *Staphylococcus aureus*: a new threat for hospital outbreaks. *Indian J Pathol Microbiol* 42:421–6.
 15. Rega AJ, Aziz SR, Ziccardi VB (2006). Microbiology and antibiotic sensitivities of head and neck space infections of odontogenic origin. *J Oral maxillofac Surg* 64:1377–80.
 16. Poeschl PW, Spusta L, Russmueller G, Seemann R, Hirsch A, Poeschl E, Klug C, Ewers R (2010). Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. *Oral Surg Oral Med Oral Pathol Oral Radio Endod* 110(2):151-6.
 17. Al-Somat M, Al-Adhal A, Ghanem N, Al-Moyed K, Shobit, AL-Kamarany MA. Susceptibility of Clinical Bacterial Isolates and Control Strains to Nigella Sativa Oil. *British biomedical bulletin*;2014(2):95-103.

Table 1. Antimicrobial resistance phenotypes of isolates of methicillin-sensitive and resistant staphylococci from pus aspirates of Deep Neck Infections

Antibiotics	MSSA isolates (n=23)	MRSA isolates (n=3)
	Resistance (%)	Resistance (%)
Penicillin	100	100
Cefazolin	0	100
Cephalexin	4.3	100
Gentamicin	4.3	0
Tobramycin	8.6	0
Netilmycin	0	0
Amikacin	0	0
Chloramphenicol	0	0
Rifampicin	0	33.3
Tetracycline	0	33.3
Ofloxacin	26	66.6
Ciprofloxacin	56.5	66.6
Cotrimoxazole	69.6	100
Erythromycin	0	33.3
Clindamycin	0	0
Fosfomycin	0	33.3
Fusidic Acid	0	0
Dalfopristine / Quinupristine	0	0
Vancomycin	0	0
Teicoplanin	0	0
Linezolid	0	0

Table 2. Phage patterns among the 14 typeable isolates of methicillin-sensitive staphylococci.

Phage group	No. of isolates	Phage pattern *	
I	3	29/52/52A/79/80	(3)
II	0	-	-
III	2	6/42E/47/53/54	(2)
Non -allocated group	0	-	-
Mixed group	9	29/52/52A/79/80/84/96	(1)
		29/52/52A/79/80/6/42E/47	(1)
		29/52/52A/79/80/6/47/53	(1)
		29/52/79/80/6/42E/47/53	(1)
		29/52/52A/79/80/6/42E/47/53/54	(4)
		3A/3c/6/42E/47/53/54/77/84/94/96	(1)
Total		14	

* Numbers in parenthesis indicate the number of isolates demonstrating a particular phage Pattern.