Research article

Teicoplanin non-susceptible coagulase-negative staphylococci in a large Australian healthcare network: Implications for treatment with vancomycin

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Abstract

Introduction and Objectives: Coagulase-negative staphylococci (CoNS) are relatively low in virulence but some are increasingly recognized as agents of clinically important infections. Glycopeptides are the drugs of choice for treatment of methicillin-resistant CoNS infections. Our aim was to analyse the susceptibility profile of CoNS in our healthcare network from 2010-2012.

Methods: All CoNS with susceptibility results were analysed as two groups; teicoplanin-susceptible (Teico-S) and non–susceptible (Teico-NS). Analysis included results of other antistaphylococcal antibiotic susceptibilities, sample type (sterile, non-sterile), species and patient location (intensive care unit (ICU) vs non-ICU).

Results: Of the 1510 CoNS isolates with susceptibility results, 109 (7.2%) were non-susceptible to teicoplanin. Teicoplanin non-susceptibility was associated with non-susceptibility to ≥ 3 antistaphylococcal-antibiotics, detected more frequently from sterile samples compared to non-sterile samples and from ICU compared to ward patients. *Staphylococcus epidermidis* was the most common species recovered and was more likely to be Teico-NS.

Conclusions: Teicoplanin non-susceptibility is associated with multi-resistance to ≥3 antistaphylococcal antibiotics. Clinicians should be aware that vancomycin resistance may be selected from Teico-NS strains.

Keywords: Coagulase-negative staphylococci, Teicoplanin, Vancomycin

Introduction

Coagulase-negative staphylococci (CoNS) comprise about 30 species of which around half are normal flora of humans. *Staphylococcus epidermidis* is commonly identified as an agent of clinically important infections due to CoNS.[1]

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The major risk factor for CoNS infections is the presence of implanted biomedical devices such as catheters, prosthetic joints, cardiac pacemakers and CSF shunts.[2] Some CoNS are able to colonise polymer surfaces by the formation of a thick, adherent, multilayered biofilm which can interfere with host defense mechanisms such as opsonophagocytosis.[3]

Over the past few decades, the significance of CoNS has increased due to the growing number of device implantations and immunocompromised patients. CoNS are now the second most common cause of prosthetic valve endocarditis after *S. aureus* and one of the most frequent pathogens isolated from deep-seated prosthetic implant infections.[4,5] CoNS have also become one of the most common causes of bacteraemia in immunosuppressed patients.[2]

Treatment of CoNS infections can be challenging owing to the frequent presence of foreign materials and increasing antimicrobial resistance. Resistance to methicillin in CoNS is very common among isolates recovered from hospitalized individuals.[6] Glycopeptides are usually the drugs of choice for treatment of methicillin-resistant CoNS infections. However, CoNS have become increasingly resistant to glycopeptides such as teicoplanin.[7] Moreover, vancomycin resistant subpopulations have also been identified within the teicoplanin non-susceptible group of CoNS.[8]

We reviewed the antibiotic susceptibilities of CoNS over a 3-year period, with the main emphasis being teicoplanin susceptibility versus non-susceptibility. This data regarding antibiotic resistance of CoNS may have an influence on the choice of empirical treatment regimens.

Materials and methods

Setting

This study was conducted at Monash Health; a large healthcare network with over 2000 acute and sub-acute inpatient beds located over five geographically distinct sites. Data was collected on all CoNS with susceptibilities performed by the microbiology laboratory from 1st January 2010 to 31st December 2012. The susceptibility testing was done only for clinically significant cases eg. clinician's request, presence of central line, and multiple positive blood cultures. Susceptibility testing was performed on urine if there was a raised WBC, a heavy pure growth, and/or catheter samples were obtained from symptomatic patients. Other non-urinary samples were tested for susceptibility if CoNS were present in pure growth or from a sterile site with a foreign body.

Annual reports for teicoplanin and vancomycin usage rates at Monash Health were extracted for 2010-2012 (personal communication) from the National Antimicrobial Utilisation Surveillance Program (NAUSP), for the two acute hospital sites (Monash Medical Centre (MMC) and Dandenong Hospital (DH) and their associated 14 and 26 bed intensive care units (ICUs).[9] Rates were expressed as defined daily doses (DDD) per 1,000 occupied bed days (OBDs).

Microbiology

Gram positive cocci were presumptively identified as CoNS by standard laboratory methods (Gram stain, colony morphology, catalase and coagulase tests). Isolates were further identified to species level using Vitek 2 GP card (BioMerieux Inc., Durham, NC) or BBL Crystal Gram

positive identification system for species level identification (BD, Sparks, USA). If identification to species level fell below 85% confidence, staphylococcal isolates were simply referred to as CoNS. Urine CoNS isolates were screened with a novobiocin disc to identify *S. saprophyticus* and if sensitive, the isolates were not further identified.

Raw data was extracted directly from the Vitek 2 Advanced Expert System (AES, BioMerieux Inc., Durham, NC) and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines for all antistaphylococcal antibiotics (oxacillin, rifampicin, daptomycin and vancomycin) except for fusidic acid in which the Comité de l′ Antibiogramme de la Société Française de Microbiologie (CA-SFM) criteria was used. Two different Vitek susceptibility cards had been used during this period: P579 GPS card was used prior to 15th October 2010 whereas P612 GPS card was used after this date and contained additional wells for the antibiotic daptomycin. Teicoplanin minimum inhibitory concentrations (MICs) were categorized into two groups; teicoplanin susceptible (Teico-S, teicoplanin MIC 4 or $\leq$ 8 μg/ml) and non–susceptible (Teico-NS, teicoplanin MIC $\geq$ 16 μg/ml).

Sample site
Clinical sample sites were categorised as sterile (blood, tissue, body fluids including cerebrospinal fluid (CSF) and peritoneal fluid), and non-sterile (urine and swabs obtained from superficial wounds or eyes). Patient location was collected from the patient information system and categorised as ICU or non-ICU.

Repeat samples originating from the same patients and site within 14 days were excluded.

Analysis
Baseline comparisons between the type of antibiotics, sample site and patient location were made using chi-square (χ^2) Fisher's exact analysis with Stata 12 (StataCorp, College Station, TX, USA).

Results

Sample site
Susceptibility testing was performed on 1510 CoNS, of which 715 Teico-S and 85 Teico-NS were from sterile site isolates and 686 Teico-S and 24 Teico-NS were from non-sterile sites.

Of the CoNS tested, 53% (800/1510) were from sterile sites with over half originating from blood (55.3%), followed by tissue (21.9%) and fluid (18.6%) respectively. Most isolates from non-sterile samples were from urine specimens (678/710, 95.5%).

Teicoplanin non-susceptibility was significantly higher from sterile site isolates (10.6%) compared to non-sterile site isolates (3.4%) (p<0.001).

Species identification
Of the 1510 isolates tested, 941 (62%) were identified to species level. Seventeen different species were identified, with *S. epidermidis* predominating (43.3%). There was a significantly

greater number of *S. epidermidis* in the Teico-NS group compared to Teico-S group (p<0.001) (Table 1).

Table 1: Teicoplanin susceptibility rate by species (speciated isolates only)

Species	Teicoplanin Susceptible (n)	Proportion of Teico-S CoNS (%)	Teicoplanin Non-Susceptible (n)	Proportion of Teico-NS CoNS (%)
S. auricularis	6	0.7	0	0
S. capitis	100	11.6	0	0
S. chromogenes	1	0.1	0	0
S. cohnii	13	1.5	0	0
S. epidermidis	332	38.5	75	96.2
S. haemolyticus	53	6.1	1	1.3
S. hominis	57	6.6	2	2.3
S. intermedius	6	0.9	0	0
S. kloosii	2	0.2	0	0
S. lentus	8	0.9	0	0
S. lugdunensis	24	2.8	0	0
S. saprophyticus	212	24.6	0	0
S. schleiferi	2	0.2	0	0
S. sciuri	3	0.4	0	0
S. simulans	12	0.4	0	0
S. warneri	28	3.2	0	0
S. xylosus	4	0.5	0	0
Total	863		78	

Antibiotic susceptibilities

Table 2: Antibiogram for CoNS by teicoplanin susceptibility

Antibiotic/s	Teico-NS group (n=109)		Teico-S group (n=1401)		P value
	Number	%	Number	%	
Oxacillin NS	98	90.7	982	70.0	<0.001
Rifampicin NS	37	34.3	70	5.0	<0.001
Fusidic Acid NS	81	75.0	346	24.7	<0.001
Vancomycin NS	4	3.7	1	0.1	<0.001
Daptomycin NS	3	3.7	43	3.3	0.56
NS ≥ 3 antibiotics*	43	39.8	62	4.4	<0.001

***** Oxacillin, Rifampicin, Fusidic Acid, Vancomycin NS non susceptible

Overall, of the 1510 CoNS tested, 7.2% were non-susceptible to teicoplanin, 71.5% to oxacillin, 7.1% to rifampicin, 0.3% to fusidic acid and 0.3% to vancomycin. Of the 1410 CoNS tested against daptomycin 3.3% were non susceptible. As shown in Table 2, Teico-NS isolates were significantly more likely to be non-susceptible to rifampicin, fusidic acid, vancomycin and to ≥ 3

antistaphylococcal antibiotics compared to the Teico-S group (p<0.001). Of the isolates from ICU, 13.7 % were found to be Teico-NS compared to 6.2 % of the non-ICU isolates (p<0.001). There was no significant difference in teicoplanin resistance between the two geographically distinct ICUs (p=0.43).

Rising Teicoplanin MIC with prolonged exposure to vancomycin

There were five patients who had the same CoNS repeatedly but with rising MIC against teicoplanin (Table 3). All these patients were treated with vancomycin.

Table 3 : Patients with repeated isolation of same CoNS species associated with a rise in Teicoplanin MIC

No	Species	Specimen	Isolate	Teicoplanin MIC (µg/ml)	Days between first and second isolate	Antibiotic duration (days) and mode of administration	Underlying illness
1	*S. epidermidis*	Peritoneal fluid	First Second	4 8	77	21 (IP)	Renal failure
2	*S. epidermidis*	Blood culture	First Second	4 **16**	61	17 (IV)	Acute lymphoblastic leukaemia
3	*S. capitis*	Blood culture	First Second	0.5 8	25	11 (IV)	Rectal cancer
4	*S. epidermidis*	Peritoneal fluid	First Second	2 8	207	14 (IP), 31 (IV)	Renal failure
5	*S. epidermidis*	Blood culture	First Second	2 **16**	324	9 (IV)	Lymphoma

All patients were treated with vancomycin and isolates had vancomycin MIC of 1-2 µg/ml. Bold= Non-susceptible. IP= Intra-peritoneal, IV=Intravenous

Vancomycin and Teicoplanin non-susceptible isolates

There were four patients with CoNS (all tissue specimens) which were not susceptible to teicoplanin, vancomycin or daptomycin. All had received antibiotic treatment with cefazolin or ceftriaxone prior to infection with CoNS, while only two had been treated with vancomycin (Table 4)

Antimicrobial usage

NAUSP specific data for glycopeptides showed an average of 30 DDD/1,000 OBDs at MMC and 34 DDD/1,000 OBDs at DH. These two hospitals provided over 70% of acute care at our healthcare network. Usage of vancomycin (160 MMC vs 150 DH) and teicoplanin (10 MMC vs 20 DH) and DDD/1,000 OBDs was similar in the 2 ICUs. Non-ICU utilisation rates for vancomycin were (26 MMC vs 30 DH) and teicoplanin (<1 MMC vs 2 DH) DDD/1,000 OBDs.

Table 4: Details of patients with Vancomycin non-susceptible CoNS

Patient	Age	Gender	Specimen and history	Species	Vancomycin MIC(µg/ml)	Teicoplanin MIC(µg/ml)	Daptomicin MIC(µg/ml)	Prior antibiotic treatment
1	75	M	Sternal Tissue Infection post heart bypass surgery	*S. epidermidis*	16	≥ 32	Not done	vancomycin cefazolin
2	54	M	Chest Wall Tissue Sternotomy reconstruction post wound breakdown	CoNS	≥ 32	≥ 32	≥ 8	vancomycin ceftriaxone piperacillin/ tazobactam
3	43	F	Right Wrist Tissue Burns to hands debrided	CoNS	≥ 32	≥ 32	≥ 8	cefazolin
4	66	F	Left Arm Tissue Iliac bone graft Orthopaedic revision	*S. epidermidis*	≥ 32	≥ 32	≥ 8	cefazolin amoxicillin/ clavulanate

CoNS= Coagulase negative staphylococci (no further speciation was attainable)

Discussion

This study aimed to analyse the susceptibility profiles of CoNS, with emphasis on teicoplanin non-susceptibility and its relationship to vancomycin in our multi campus healthcare network.

There is limited available local information in the literature concerning CoNS and glycopeptide resistance. Ma *et al* reported on 745 isolates using microbroth dilution and found that there had been an increase in CoNS Teico-NS from 4.5% in 2008 to 6.7% in 2009, while all isolates remained susceptible to vancomycin.[8] Other studies have reported reduced susceptibility overall to glycopeptides at 5.1% and teicoplanin at 8.1%.[2] Similarly, our study showed 7.2% of isolates to be Teico-NS with vancomycin resistance at 0.3%.

Prolonged exposure to vancomycin is a possible risk factor for the development of resistance to teicoplanin.[10] It has been shown that stepwise exposure to vancomycin can induce resistance, particularly in *S. haemolyticus* and *S. epidermidis*.[7] This is possible since the gene for teicoplanin resistance is co-located with another antibiotic resistance gene/s.

The most frequent species detected in Teico-NS CoNS in our study was *S. epidermidis* (96.2% compared to 38.5% of Teico-S CoNS). Reduced susceptibility to glycopeptides has previously been most commonly seen in *S. haemolyticus* but this was quite rare (0.6%) in our study collection.[8,11,12] Other species which have been reported to be glycopeptide non-susceptible include *S. lugdunensis*, *S. capitis* and *S. simulans*.[2,4,6,13] However, none of these species were identified as Teico-NS in the current study. CoNS from patients in ICU were more likely to be

Teico-NS. This could possibly be due to long-term exposure to multiple antibiotics which has selected for resistant strains.

Studies using population analysis profile (PAP) testing have shown that vancomycin hetero-resistant phenotypes have been identified within Teico-NS isolates from patients who have had prolonged glycopeptide therapy.[8,14] Although the mechanism of action is not clearly known, increased cell wall thickness in these subpopulations is similar to that seen for *S. aureus* hetero-resistance when challenged with vancomycin.[10] It is not known why resistance develops sooner for teicoplanin than vancomycin and one theory proposed that teicoplanin binds more avidly to CoNS.[13]

The main limitation of our study is that it was based on review of retrospective data and the clinical significance of the studied CoNS isolates had not been determined. Over a third of all CoNS (predominantly urines) were not further identified to species level by standard biochemical methods. Identification using matrix-assisted laser desorption ionization-time of flight mass spectrometry has only recently become available at our hospital site. Confirmation of true teicoplanin MIC using Etest was not conducted on Teico-NS isolates and we have relied on the three concentrations (4ug/ml, 8ug/ml, 16ug/ml) tested by Vitek. Isolates were not available for PAP testing and hence we are unable to confirm if hetero-resistant populations may be present. Molecular strain typing was not undertaken and it is possible that the same clonal strains of CoNS may be circulating in high-level care wards such as ICU. Importantly, some of our results may be skewed with regard to CoNS from ICU as such isolates are more likely to be investigated rather than considered to be non-significant.

Further surveillance is required to examine the prevalence of glycopeptide non-susceptible CoNS at other institutions. The mechanism of action needs to be clearly determined by studying cell wall membrane proteins and antibiotic binding sites. The implications for treatment with vancomycin requires further investigation and for the time being clinicians should be aware that potentially vancomycin resistance may be selected from teicoplanin non-susceptible strains.

Conclusion

In conclusion, for serious CoNS infections, susceptibility testing should be performed including testing for glycopeptides. This will allow for more appropriate antibiotic treatment of these difficult to treat infections

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