De novo daptomycin non-susceptible enterococci causing urinary tract infection: a study from north India

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Abstract

Introduction: Daptomycin is a bactericidal agent active against vancomycin resistant enterococci (VRE) which are emergent nosocomial uropathogens. There is limited data available on daptomycin non-susceptible enterococci (DNSE) in India. Herein we describe the emergence of de novo DNSE causing urinary tract infections (UTI) from India.

Materials and Methods: We prospectively screened, consecutive enterococci (n=140) grown in significant numbers ($\geq 10^5$cfu/ml) from urine cultures in hospitalized patients (n=12434) over 6 months. Isolates with daptomycin minimum inhibitory concentration (MIC) >4µg/ml by E-test and no history of daptomycin exposure were defined as DNSE. Colonization and symptomatic UTI was defined as per the Centre for Disease Control and Prevention guidelines.

Results: Prevalence of DNSE was 12.1% (17/140) and all were *E. faecium*. In 6 cases (35.2%) DNSE isolates were colonizers while 11(64.71%) were from UTI cases. Urosepsis occurred in 4 cases, of which 3 died. History of immunocompromise, recent urogenital surgery and indwelling per-urethral catheter were present in 47%, 58.8% and 64.7% cases respectively. Exposure to third generation cephalosporin and metronidazole was seen in nine (52.9%) and 3 (17.65%) cases respectively, while one patient each had vancomycin and teicoplanin exposure. Daptomycin MIC range was 6 to >256 µg/ml. Resistance to ciproflocaxin, amoxicillin, high level gentamicin, tetracycline, nitrofurantoin and vancomycin was seen in 100%, 94.1%, 88.2%, 52.9%, 41.1% and 23.5% respectively. All DNSE were susceptible to linezolid.

Conclusions: A high prevalence of DNSE warrants further case control studies, molecular and epidemiological studies to elucidate the risk factors, molecular mechanisms of resistance and epidemiological origin of these isolates.

Keywords: De novo, Daptomycin, Enterococci, UTI, India.

Introduction

Daptomycin, a cyclic lipopeptide is a bactericidal agent active against many gram-positive organisms including vancomycin resistant *Staphylococcus aureus* (VRSA), vancomycin intermediate *Staphylococcus aureus* (VISA), methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE). The treatment of Enterococcal infections presents a challenge primarily because of limited therapeutic options due to the widespread prevalence of strains with resistance to multiple antibiotics. Daptomycin is currently licensed by United States Food and Drug Administration (U.S. FDA), for use in S. aureus blood stream infections, particularly infective endocarditis and complicated skin and soft tissue infections and has in addition, been found effective and safe for treating urinary tract infections (UTIs) due to VRE.[1] Daptomycin exhibits good activity against *enterococci* as evidenced by, large scale susceptibility studies, irrespective of the susceptibility to other agents.[2] Although the precise mechanism of action of this agent is incompletely understood, the lack of cross resistance to other anti bacterial agents suggests that it acts distinctly from other antimicrobials and it is hypothesized to exert its effect primarily by disrupting the bacterial cell membrane. Despite its utility, emergence of resistance to daptomycin during treatment is a well described phenomenon that threatens its use in clinical practice, and further limits therapeutic options against VRE. De-novo resistance to daptomycin among VRE without prior exposure was first reported by Lesho et al[3] in 2006 in a case of endocarditis, followed by Fraher et al[4] who reported a blood culture isolate from a patient of Crohn's disease. Recently, Kelesidis et al[5,6] reported de-novo daptomycin resistance in urinary isolates. Herein, we report the de-novo emergence of urinary daptomycin non susceptible enterococci (DNSE) from a tertiary care centre in India and discuss their clinical and microbiological aspects. To the best of our knowledge, this study describes the largest series of de novo urinary DNSE isolates reported so far and represents the first case series from India.

Materials and Methods

Sample Details: This prospective study was carried out at Postgraduate institute of medical education and research (PGIMER), Chandigarh, a north Indian tertiary care referral centre. It was carried out over a period of 6 months from January 2013 to June 2013. A total of 12434 urine samples from hospitalized patients clinically suspected of UTI were evaluated and all consecutive isolates (n=140) with culture growth in significant numbers ($\geq 10^5$ colony forming units (CFU) per ml) were included in this study.

Identification of Isolates: Species identification of the isolates was done using conventional biochemical tests. The identification was confirmed by matrix assisted laser desorption-time of flight (MALDI-TOF) by Bruker Daltonics, Germany and the mass spectra generated were analyzed by MALDI Biotyper 3.0 software.

Antimicrobial Susceptibility Testing: The daptomycin minimum inhibitory concentrations (MIC) was estimated by preparing the bacterial inoculum in cation adjusted Muller-Hinton broth (BD Difco, Gurgaon, India) supplemented with calcium (50 µg/ml) followed by Etest (bio-Merieux, Durham, NC, USA). Isolates with a MIC of > 4µg/ml were categorized as daptomycin non-susceptible *Enterococci* (DNSE) as per the Clinical and Laboratory Standards Institute (CLSI), 2014 interpretative criteria.[7] The isolates were defined as de-novo DNSE if there was no prior exposure to daptomycin. Antimicrobial susceptibility testing was also done for amoxycillin (10 µg), high level gentamicin (120 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg), tetracycline (30 µg), teicoplanin (30 µg), vancomycin (30 µg), and linezolid (30 µg) by Kirby Bauer disc diffusion technique and interpreted as per CLSI guidelines. *Enterococcus faecalis* ATCC 29212 was used as the control strain.

Clinical Details: The clinical records of all the enrolled cases were retrieved and analysed. Cases of colonization (asymptomatic bacteriuria) and symptomatic UTI (sUTI) were defined by using the criteria of CDC/ National Healthcare Safety Network, 2013.[8] Briefly, patients were labeled to have sUTI if at least one of the following was present: fever (38ºC), frequency, dysuria, urgency or suprapubic tenderness with no other identifiable cause was seen in addition to the positive urine culture. If there were no signs and symptoms suggestive of UTI, the cases were labeled as asymptomatic bacteriuria and isolates labeled as colonizers.

Discussion

Recent years have seen a rise in DNSE as agents of hospital associated infections; however their epidemiology is yet to be completely understood. According to a recent systematic review, including 23 studies a DNSE prevalence of less than 2% was observed with higher prevalence (range 10%–19%) noted from Asian and European countries.[6] We describe a prevalence of 12.1% (17 cases) for DNSE with no prior history of daptomycin usage, suggesting their de-novo emergence in our clinical settings. However, the relatedness of the isolates was not performed in the present study; the high prevalence reported may be due to circulation of clonally related isolates in the institution. In a study by Storm et al DNSE were identified in 25 patients from different samples (blood, peritoneal fluid, urine etc) of which 40 % were de novo-DNSE.[9] They described fourteen cases from urine of which 50% were associated with true infection and a rise in the isolation of DNSE in cases of infection versus colonization was noted over the 5 year study period. In our study we found the majority of 64.7% (n=11) cases to due to true infection (sUTI) with DNSE colonization in 35.3% patients. Thus, the presence of DNSE in urine may not always represent infection and it is imperative to determine the clinical significance of these isolates.

All DNSE isolates recovered in our study were *E. faecium.* Similarly, *E. faecium* has been reported in other studies, as the predominant species with decreased susceptibility to daptomycin with daptomycin non susceptible *E. faecium* ranging from 70-88% [5,6,9] The MIC's for the DNSE isolates in the present study range from 6 to >256 µg/ml. Interestingly, in a recent study by Kelesidis et al, 2013 eleven isolates of DNSE were recovered of which 3 were de-novo DNSE.[5] All the de-novo DNSE were identified as *E. faecalis* and exhibited a lower median daptomycin MIC than the *E. faecium* isolates (6 versus 28 mg/ml). *E. faecalis* is known to be more prevalent than *E. faecium* in the community setting and this could explain the higher prevalence of *E. faecium* in our study as since 88.2% of our patients had nosocomial UTI. *E. faecium* strains display a greater degree of antimicrobial resistance to multiple antibiotics and this could be the reason why we observed a higher MIC range for de-novo DNSE in our study.

Amongst other antimicrobial agents tested, vancomycin resistance is commonly reported with DNSE (72-100%).[10,11] We reported a 23.5% concomitant resistance to vancomycin in our study. Most studies do not report daptomycin MIC for vancomycin-susceptible enterococci (VSE) however this may result in an under estimation of the incidence of DNSE isolates. There is also evidence of high prevalence of ampicillin resistance in DNSE isolates (72%- 80.6 %).[5,9] For treatment of DNSE infections, since ampicillin can alter the susceptibility to daptomycin by acting on the surface charge of the organism, combination therapy of daptomycin with ampicillin can be another potential alternative for the treatment of DNSE isolates.[12] Though we did not test for ampicillin, 94.12% of DNSE isolates were resistant to amoxicillin in our series. Due to the high prevalence of resistance to ciprofloxacin, tetracycline, gentamicin and nitrofurantoin in our study linezolid could be a very good alternative as all DNSE were susceptible.

There is scant literature on the possible risk factors that are associated with DNSE. Prior exposure to daptomycin, immunosuppression, history of prior hospitalization and concomitant gastrointestinal surgeries have been reported to have an association.[9,13] We observed that surgical interventions and presence of indwelling urinary catheter were associated with urinary isolates of DNSE, present in 58.8% and 64.7% of cases respectively. Higher isolation of DNSE isolates

in immunosuppressed patient recorded in this study is similar to the study by Kelesidis et al[5,6] who reported 72.7% to 77.8% of DNSE isolates from such individuals.

Prior use of antimicrobials such as vancomycin, cephalosporins or anti-anaerobic agents has been found to promote the VRE emergence and may potentially have an association with the DNSE. It has been recently suggested that interplay between bowel anaerobes and enterococci could be responsible for the dissemination of resistance to daptomycin. Various anaerobes are intrinsically non susceptible to daptomycin, raising the possibility of a genetic exchange between the *enterococci* and anaerobes.[14] Anti-anaerobic treatment may induce stress responses in anaerobes in our gut promoting horizontal gene transfer and development of DNSE.[13] In our study we noted previous exposure to metronidazole in 17.6 % of the DNSE isolates. We also noted a 53% prevalence of cephalosporin exposure in the *de novo* DNSE isolates from our study. Nevertheless, the role of genetic transfer of resistance encoding genes between daptomycin non susceptible bacteria and *enterococci* in our gut remains speculative, in particular for *de novo* DNSE isolates.

Our study is the largest report of urinary isolates of DNSE and highlights the significance of this pathogen in sUTI. However, it is limited by the observational study design and the lack of a comparison group. Though majority of our strains appeared to be nosocomially acquired, some were definitely community acquired and DNSE acquisition from food products and zoonotic transmission cannot be ruled out.[15] Although, case–control studies are required to better define the risk factors associated with emergence of DNSE, our study emphasizes the need for daptomycin MIC breakpoints evaluation for urinary isolates of *Enterococcus*. The presence of de- novo DNSE causing UTI in hospitalized patients is indeed alarming and merits continuous and stringent monitoring.

Results

A total of 17 (12.14%) DNSE isolates were isolated and all were identified as *E. faecium*. The demographic and clinical details of these 17 cases are shown in table 1. The study included a total of 8 male and 9 female patients with a median age of 30 years (range 3 days – 76 years). Notably, in majority of 64.7% (n=11) cases a diagnosis of sUTI was made while DNSE were colonizers in 6 (35.3%) patients.

Previous exposure to third generation cephalosporins and metronidazole was observed in 9 and 3 patients respectively while exposure to vancomycin and teicoplanin was noted in one patient each. On the evaluation of risk factors for DNSE we observed that invasive interventions such as surgery or percutaneous nephrostomy (PCN) within three months prior to isolation of DNSE were present in 10 (58.8%) patients. Remarkably, no patient had history of repeated hospital admission and only three had stayed in intensive care unit (ICU). Eleven (64.71%) patients had indwelling per-urethral catheter (PUC) and five (29.42%) patients had a central venous catheter in situ at the time of sample collection. In the majority of patients (n=15, 88.2%), DNSE appeared to be nosocomially acquired with average length of hospitalization before isolation of the first DNSE isolate being 13.6 days (range 2-41 days). In 2 (11.8%) patients, DNSE were recovered in less than two days following hospital admission suggesting community acquired UTI.

In patients with sUTI, vancomycin was added in two cases and nitrofurantoin in another case after the isolation of DNSE. In rest of five cases of sUTI, no specific treatment targeted to DNSE was given. No additional antibiotic was given to six cases in which DNSE was found to be colonizers. The average hospital stay was 25.3 days (range 3-56 days).Urosepsis was noted in 4 (36.4%) patients with sUTI in which a high mortality of 75% was seen. No mortality was noted amongst the UTI cases without sepsis and amongst colonizers.

The antimicrobial susceptibility results are shown in Table 2. The MIC range of daptomycin, teicoplanin and vancomycin was 6 to >256 µg/ml, 0.12 to 96 µg/ml and 0.5 to >256 µg/ml respectively. Resistance to ciprofloxacin, amoxicillin, high level gentamicin, tetracycline and nitrofurantoin was seen in 100, 94.1, 88.2, 52.9, and 41.1 % of the isolates, respectively. Four (23.53%) isolates had concomitant resistance to vancomycin and teicoplanin. Non-susceptibility to daptomycin was independent of susceptibility to vancomycin. Highest activity was shown by linezolid (100% susceptible) followed by teicoplanin (76.47% susceptible).

Table 1:

Table 1. Epidemiological, clinical and microbiological characteristics of 17 de novo urinary DNSE* isolates from India

Patient no., age (y), sex	UTI*/ colonisation	Recent surgery (postsurgical day of DNSE isolation)	Catheterizatio, (postcatheterization day of DNSE isolation), other lines	Hospital day of DNSE isolation	Comorbid conditions	Antibiotic (days of therapy prior to DNSE isolation)	Treatment Post DNSE	Other pathogens isolated at DNSE site	ICU* stay days)	Total Hospital stay	outcome
1/ 52/ M	Coloniser	Craniotomy (21)	PUC*(21), CV* line (21)	22	Nil	Amikacin (10) Meropenem (20)	Nil	Nil	Nil	38	Improved
2/ 76/ M	UTI	B/LPCN* (4)	PUC (4)	4	Hydronephrosis	Imipenem (4)	Imipenem (11)	Nil	Nil	11	Improved
3/ 16/ F	UTI	Nil	CV line (2)	2	Gall stones Connective tissue disorder	Ceftriaxone (3) Pip-taz (2)	Imipenem (12) Vancomycin (4) Levofloxacin (23) Metronidazole (23)	NFGNB* (blood culture) E coli (urine)	20	52	Improved
4/ 50/ F	UTI with sepsis	Nil	PUC (45), CV line (45)	28	Old treated Astrocytoma Old cardiovascular accident	Colistin (10) Sulbactam (7) Capsofungin (3)	Colistin (12) Sulbactum (10) Caspofungin (14) Imipenem (5)	Acinetobactersp (tracheal aspirate) Enterobactersp (blood, pleural fluid and tracheal aspirate)	50	52	Death
5/ 40/ F	UTI	Nil	PUC (10), CV line (10)	41	Diabetes mellitus Steroids	Colistin (2) Imipenem (14) Pip-taz (9) Teicoplanin (14) Vancomycin (11)	Pip-taz (14) Minocycline (3)	Acinetobactersp (tracheal aspirate)	1	44	Improved
6/ 32/ M	UTI	Craniotomy with lobectomy (9)	Nil	21	Nil	Ceftriaxone (9)	Ceftriaxone (21)	Nil	Nil	26	Improved
7/ 3 days/ M	UTI with sepsis	Nil	PUC (3)	3	B/L hydronephrosis	Amikacin (3) Cefotaxime (3)	Amikacin (9) Ceftriaxone (12) Nitrofurantoin (8)	Nil	Nil	12	Death
8/ 10 months/ F	Colonisation	Nil	Nil	12	Pneumonia	Amikacin (6), Ceftriaxone (6)	Nil	Nil	Nil	12	Improved
9/ 30/ F	Colonisation	PCN (14)	PUC (10)	9	Acute renal failure, deep vein thrombosis Pulmonary thromboembolism	Nitrofurantoin (15) Ciprofloxacin (5)	Nil	E coli (urine)	Nil	16	Improved
10/ 12/ M	Coloniser	Segmental resection (4)	Nil	4	Nil	Cefoperazone – sulbactum (4)	Cefoperazone – sulbactum (7)	Nil	Nil	5	Improved

11/ 45/ M	UTI with sepsis	Ethemoidectomy (4)	PUC (7)	9	Nil	Augmentin (9)	Augmentin(14) Amikacin (5)	Nil	Nil	16	Improved
12/ 52/ F	UTI	Bladder repair (5)	PUC, SPC* (15), CV line (15)	9	Radiation therapy	Cefoperazone-sulbactam (10) Amikacin, (19) Metranidazole (8)	Vancomycin (7)	E.fecium (wound)	Nil	24	Improved
13/ 2/ M	UTI with sepsis	Extraventricular drain surgery (1)	PUC (1)	1	Septic shock	Amikacin (1), Ceftriaxone (1)	Amikacin (3), Ceftriaxone (3) Acyclovir (3)	Nil	Nil	3	Death
14/ 19/ F	Colonisation	Nil	Nil	5	Nil	Ceftriaxone (5) Metronidazole (3)	Ceftriaxone (6)	E. coli (urine)	Nil	13	Improved
15/ 14/ M	UTI	PCN (1)	PUC (1)	1	Renal stone with hydroureteronephrosis Acute renal failure	Nil	Cefepime (17) Metronidazole (4)	P.aeruginosa (PCN)	Nil	56	Improved
16/ 12/ F	UTI	Spleenectomy (6)	PUC (11)	26	Continuous ventilator support, Steroids	Ceftriaxone (14) Cloxacillin (14) Amikacin (6) Piptaz (9) Metranidazole (6)	Imipenem (5) Nitrofurantoin (5)	Acinetobacter sp. (blood)	Nil	36	Improved
17/ 45/ F	Coloniser	Nil	Nil	9	Steroids	Pip-taz (7)	Nil	Nil	Nil	15	Improved

Table 2: Antimicrobial susceptibilities of Denovo Daptomycin Nonsusceptible Enterococcal isolates from 17 patients to antimicrobial drugs with activity against Enterococcus spp

Amox (10µg)	Cipro (5µg)	HLAR* (120µg)	Nitro (300µg)	Vanco (30µg)	Teico (30µg)	Tetra (10µg)	Dapto MIC§ (µg/ml)	Vanco MIC (µg/ml)	Teico MIC (µg/ml)
R	R	R	S	S	S	R	6	1	0.38
R	R	R	S	S	S	R	12	1.5	0.12
R	R	S	S	S	S	R	6	0.5	0.19
R	R	R	R	R	S	S	32	>256	1.5
R	R	R	R	I	R	S	>256	8	96
R	R	R	R	I	S	S	>256	8	0.5
R	R	R	S	S	S	R	6	0.5	0.25
R	R	R	S	R	I	R	6	>256	24
R	R	R	S	S	S	R	6	0.75	0.5
R	R	R	R	S	S	S	6	1.5	0.25
R	R	R	R	S	S	S	8	1	0.75
R	R	R	R	S	S	S	6	1	0.5
R	R	R	S	S	S	R	8	2	0.5
R	R	R	R	S	S	S	8	1	1
S	R	S	S	R	R	R	192	>256	32
R	R	R	S	R	R	S	32	>256	
R	R	R	S	S	S	R	6	1	

*Amox- Amoxicillin, Cipro- Ciprofloxacin, HLAR- high level aminoglycoside (gentamicin) resistance, Nitro- Nitrofurantoin, Vanco- vancomycin, Linz- linezolid, Teico- teicoplanin, Tetra- tetracycline, Dapto- Daptomycin, Vanco-Vancomycin, Teico- Teicoplanin § MIC= Minimum Inhibitory Concentration, R= Resistant, S= Senitive; Vancomycin S≤4 mg/L, I=8–16 mg/L, R≥32; teicoplanin S≤8 mg/L, I=16 mg/L, R≥32 mg/L; daptomycin S≤4 mg/L.

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