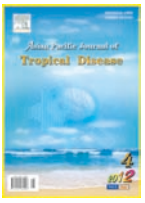
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage:www.elsevier.com/locate/apjtd



Document heading doi: 10.1016/S2222-1808(12)60060-7

Surveillance of multidrug resistance of two Gram-positive pathogenic bacteria in a teaching hospital and *in vitro* efficacy of 30 ethnomedicinal plants used by an aborigine of India

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ARTICLE INFO

Article history:
Received 11 April 2012
Received in revised form 14 April 2012
Accepted 2 May 2012
Available online 28 August 2012

Keywords:
Plants
Ethnobotanical information
Pathogenic bacteria
Gram-positive bacteria
Multidrug resistant strains
In vitro antimicrobial activities
Staphylococcus aureus
Enterococcus faecalis
Lantana camara
Woodfordia fruticosa
Oroxylum indicum
Phytochemical analysis
MRSA
VRSA

ABSTRACT

Objective: To record hospital- and community-acquired accounts of multidrug resistance (MDR) of two Gram-positive pathogens, *Staphylococcus aureus* (*S. aureus*) and *Enterococcus faecalis* (*E. faecalis*), by surveillance, and to evaluate antibacterial potencies of 30 plants with information on ethnomedicinal uses for infectious ailments by the aborigine Kandha tribe of Kalahandi district, Odisha (India), against both pathogens. **Methods:** Over a period of 6 months bacteria/ strains of *S. aureus* and *E. faecalis* were isolated from clinical samples in a teaching hospital and their antibiograms were ascertained using 17 antibiotics of 9 different groups. *S. aureus* strains were further tested for confirmation if they were methicillin and vancomycin resistant, similarly, *E. faecalis* strains for vancomycin resistance. Concentrated aqueous and ethanolic extracts of leaves/ barks of 30 plants were used for monitoring their antimicrobial potencies, by the agar-well diffusion method, along with qualitative phytochemical analyses. **Results:** From the surveillance, both pathogens were found MDR and it was evident that the distribution of MDR strains was more in hospital-acquired than community-acquired samples. Both aqueous and ethanolic extracts of plants, *Diospyrous melanoxylon*, *Woodfordia fruticosa* (*W. fruticosa*), *Oroxylum indicum* (*O. indicum*), *Dalbergia paniculata* and *Lantana camara* had the most significant *in vitro* controlling capacity against MDR strains of both bacteria. Further, extracts of *Holarrhena antidysenterica*, *Aspidopterys tomentosa* and *Argyreia speciosa* had moderate antibacterial activities. Ethanolic extracts of *L. camara*, *O. indicum* and *W. fruticosa* contained all the phytochemicals, alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids, which could be attributed to the recorded significant antibacterial activity. **Conclusions:** *S. aureus* strains have been found as the most widely prevailing pathogens in nosocomial settings, than in community. Plants, *L. camara*. *W. fruticosa*, *O. indicum* and *P. santalinus*, particularly could be useful for a use as complementary/ supplementary/alternative therapeutic agents against Gram-positive pathogens.

1. Introduction

Drug resistance in pathogenic bacteria has been detected since decades and multidrug resistant (MDR) strains of both Gram-negative and Gram-positive (GP) bacteria have emerged increasingly as public health perils, since many strains are almost untreatable. MDR GP bacteria are less prevalent than MDR Gram-negative ones[1], but *Staphylococcus* and *Enterococcus* spearhead among MDR GP cocci[2,3], which are considered as the important determinants of public health problems, worldwide. *Staphylococcus aureus* (*S. aureus*) causes mild to severe or potentially fatal illness. There are about 30 species of *Staphylococcus*, but the most grievous infections are caused by *S. aureus*. Indeed, the problem from its infection have been multiplied by the development of resistance to beta-lactams and a large number of antibiotics of other groups, aminoglycosides, glycopeptides, fluoroquinolones, sulfonamides, etc. By the by, MDR 'methicillin resistant *S. aureus*' (MRSA) has been considered as the superbug in the health domain, today[4]. The most common ailments caused

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Foundation Project: Supported by a Major Research Project on Botany[39-388/2010(SR)] titled 'Alternative drug search from ethnomedicinal plants of Odisha against multidrug resistant bacteria' awarded to R. N. Padhy, by University Grants Commission, New Delhi.

by *S. aureus* are impetigo, cellulitis, scaled skin syndromes and mastitis-all leading to infections of newborns from their mothers. When *S. aureus* enters the blood stream, invasive infections, bacteraemia/sepsis staphylococcal pneumonia, endocarditis, osteomyelitis and 'the toxic shock syndrome' occur; it also causes illness of the bowel, like food poisoning[5]. It was never so difficult to control all these ailments due to *Staphylococcus* earlier, but multidrug resistance, extensively drug resistance and pandrug resistance described in literature for it[6,7], with intractable, wily and ghoulish clonal complexes that land at abysmal annoyance in the health domain.

The other dominating pathogen, *Enterococcus* causes urinary tract infections (UTI) and surgical site infections. In fact, *Enterococcus faecalis* (*E. faecalis*) is a saprophytic component of the enteric flora and causes severe comorbidities from peritonitis, intra-abdominal abscess and endocarditis, when it gains a portal entry to the blood stream, as this pathogen has got remarkable adherence properties to human serum[8,9]. Belonging to group D streptococci, *E. faecalis* have been reported to have intrinsic resistance to cephalosporins, aminoglycosides, beta-lactams and vancomycin (glycopeptide). So, this pathogen has the potentiality of precipitating outraging episodes linked to gastroenteritis and UTI[10]. Enterococci were reported as the second most common cause of nosocomial infections in the US; those account for more than 9% of blood-stream infections (BSIs) in the US and Canada (rates are lower in Latin America); the highest detected rate of enterococcal UTI was reported in Canada (16.8%), followed by the US (12.5%) and Europe (11.7%)[10]. Viewed from the trenches of public health, the thunderclap-like situation of pandrug resistance in many bacteria (*Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, among Gram-negatives, and *S. aureus* as the GP, to cite the few among the cornucopia of MDR pathogens), create the damndest tumult in public health all over[13]. So, a surveillance of two GP pathogens, *S. aureus* and *E. faecalis* (causatives of high decimations) was pursued that record an eye-brow raising infection dynamics of both bacteria. And the surveillance of *P. aeruginosa* in this hospital was recently reported[12].

In this perspective, the search for complementary and alternate therapeutics becomes an obsessive quest and phyto-extracts remain a palpable source, possibly for MDR pathogens in general, not least because, the *Streptomyces*-source of antibiotics is exhausted, but phyto-compounds inherently with unbreachable barrier of complexity and being of non-microbial origin, no microbe how much genetically equipped and developed be it may, can ever over-ride these coalesced chemicals in a module, accessorized with the formal antimicrobial stewardship program. The present report embodies an attempt in this line after our first report[11], against a cohort of 11 pathogenic bacteria in a preliminary study with 20 well-known medicinal plants with ethnobotanical history; this paper records the ethnobotanical history of lesser-known 30 medicinal plants obtained from Kalahandi district (Eastern Ghats at Odisha). For hundreds of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. The antimicrobial efficacy attributed to some plants in treating diseases has been beyond belief[14]. It is estimated that tribal communities are using about 10% of all flowering plants on earth to treat various infections, although only 1% have gained recognition by modern scientists[15]. Since their popular use as remedies for many infectious diseases, searches for plants containing antimicrobial substances are frequent[16]. Plants are rich with a range of secondary metabolites such as tannins, alkaloids, flavonoids and a few more, which have been found *in vitro* to have antimicrobial properties[17]. A number of phytotherapy manuals and research journals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity on humans[18,19]. Many plants have been found to cure urinary tract infections, gastrointestinal disorders, respiratory diseases and cutaneous infections and many more[20,21]. According to the WHO, medicinal plants would be the best source for obtaining a variety of drugs[22]. These evidences contribute to support the importance of the screening of natural products for new pharmaceuticals. Thus, it was intuitive to screen a large number of lesser-known plants for helping a possible alternative therapy for treating MDR pathogens. This study records the surveillance of *S. aureus* and *E. faecalis* strains from hospital and community settings. Further antibiogram of 17 antibiotics belonging to 9 groups are described for representative isolates of both pathogens from both sources. Further, qualitative phytochemical analyses of 30 plants have been included for possibilities of linking antimicrobial activity of plants to their constituents. It is anticipated that crude phytochemicals being an array of natural compounds of non-microbial origin, it would be impossible for any genetically well-developed bacterial strain even, to win over those. Further, this study should help in distinguishing plants for their future role in the use as antimicrobials of non-microbial origin for these grisly pathogens.

2. Materials and methods

2.1. Survey work and preparation of plant extracts

Plants reported were collected from hills of Eastern range of mountains of India, in the district Kalahandi, Odisha state in February 2010. Details of survey work and preparation of plant extracts were done, as previously described[13].

2.2. Collection and processing of clinical samples

From different clinical samples (pus, swabs, urine, body fluids and blood) two pathogens *S. aureus* and *E. faecalis* were isolated from the outpatients department of Sum Hospital or community) as well as, from patients admitted into different units of the hospital. A total of 708 positive clinical samples were obtained over a period of 6 months (November 2009-April 2010).

GP cocci were cultured on nutrient agar and blood agar. On nutrient agar, butyrous, glistening, round, elevated, medium-sized colonies with golden colour (due to presence of triterpenoids or carotenoids in cell membrane), and on blood agar with yellow coloured, round and elevated colonies with beta-haemolysis seen, and those were taken as *S. aureus*. For further confirmation, the colonies were streaked on mannitol salt agar medium and were incubated at 37 ℃, for 48 h; yellow colonies were identified as *S. aureus* colonies[23]. Similarly, *E. faecalis* produced small, round,

grey-coloured gamma-haemolytic colonies on blood agar that failed to survive on nutrient agar[23]. Strain of *S. aureus* number 7443 and *E. faecalis* number 439 from Microbial Type Culture Collections (MTCC) served as reference controls.

2.3. Biochemical identifications

For pure-cultures of isolated GP cocci, catalase, oxidase and coagulase tests were performed and recorded positive, as detailed previously[13]. Catalase negative colonies were subjected to bile esculin test. The bile esculin medium contains esculin and peptone for nutrition and bile to inhibit growth of GP bacteria, other than group D streptococci and enterococci. Ferric citrate was added as a colour-indicator. Organisms, which split the esculin molecules and use the liberated glucose to supply energy, release esculin into the medium. The free esculin reacts with ferric citrate in the medium to form a phenolic iron complex, which turns the agar-slant from dark brown to black. An agar-slant that was more than half darkened within 48 h was bile-esculin positive for *E. faecalis*, alternatively non-darkening of agar was taken as the negative result[23].

2.4. Antibiotic sensitivity test

All isolated *S. aureus* and *E. faecalis* strains were subjected to antibiotic sensitivity tests with Muller-Hinton agar (MHA) medium and blood agar, respectively, by the disc diffusion/Kirby-Bauer's method, detailed previously[13], according to CLSI guidelines[24].

2.5. Detection of MRSA by chromogenic agar media test

Purified clinical isolates of *S. aureus* were streaked onto methicillin resistant *S. aureus* agar media (Hichrome-MeReSa agar media, HiMedia, Mumbai) and were incubated for 24 h. The colonies appearing blue after incubation period were detected as MRSA strains, and non-MRSA strains appeared white.

2.6. Detection of vancomycin resistant Staphylococcus aureus and vancomycin resistant Enterococcus faecalis

Screening for vancomycin resistance was done by agar screen methods on both MHA and brain heart infusion (BHI) agar (HiMedia). Vancomycin screen agar plate was prepared by an addition of 6 mg/L vancomycin to brain BHI agar and MHA. Inoculum suspension was prepared by transferring colonies from overnight growth on nutrient agar plate to sterile saline to produce a suspension that matched the turbidity of a 0.5 McFarland standard. An aliquot of 0.1 mL of the suspension was spread on vancomycin screen agar plate and was incubated for 24 h at 37 ℃. Any visible growth indicated the vancomycin resistance. In addition, *S. aureus* MTCC 7443 and the *E. faecalis* MTCC 439 were used asmethicillin/vancomycin-susceptible control strains[25].

2.7. Antibacterial activity test by agar-well diffusion method

One strain from each bacterial species showing resistance to a maximum number of antibiotics was further used for monitoring antibacterial potentiality of plants extracts, by the agar-well diffusion method, detailed previously[13]. For *S. aureus*, MHA was used whereas blood agar was used for *E. faecalis*. The extracts causing a zone of inhibition 20 mm or more were considered highly active and plants having a zone of inhibition less than 20 mm were considered moderately active. Linezolid 30 μg/mL with an average size of zone of inhibition of 20 mm and DMSO 10% with no antibacterial activity were taken as reference controls.

2.8. Preliminary phytochemical analysis

The presence of free reducing sugars was ascertained by Fehling's test[26]. An aliquot of 0.5 g of an extract was dissolved in an aliquot of 10 mL of distilled water in a test-tube and was shaken vigorously for 30 seconds, and then allowed to stand for 45 minutes. The appearance of a frothing on warming indicated the presence of saponins[26]. To a portion of the dissolved extract, a few drops of 10% FeCl3 solution were added. A green or blue colouration of the solution indicated the presence of flavonoids[27]. A lot of 500 mg of the extract was dissolved in an aliquot of 2 mL of acetic anhydride and cooled at 0 to 4℃, to which a few drops of 12 N sulphuric acid was carefully added. A colour change from violet to blue-green indicated the presence of a steroidal nucleus[27]. A lot of 0.5 g of the extract was dissolved in 5 mL of water followed by a few drops of 10% FeCl3. A blue-black, green, or blue-green precipitate indicated the presence of tannins[26]. A lot of 0.5 g of ethanol extract was stirred with an aliquot of 5 mL of 1% HCl on a steam bath and filtrated; to an aliquot of 1 mL of the filtrate, a few drops of Mayer's reagent were added, and to another aliquot of 1mL of the filtrate, a few drops of Dragendorff's reagent were added. Turbidity or precipitation in tubes due to either of these reagents indicated the presence of alkaloids in the extract[26]. To an aliquot of 10 mL of the extract an aliquot of 10 mL of copper acetate solution 1% was added and shaken vigorously and a separate green colour indicated the presence of resin[27]. An aliquot of 5 mL of each extract was mixed with an aliquot of 2 mL of glacial acetic acid (1.048 g/mL), one drop of 1% FeCl3 solution, and mixed thoroughly to which, an aliquot of 1 mL of 12N H_2SO_4 was added. A brown ring at the interface indicated the presence of glycosides[27].

3. Results

S. aureus and *E. faecalis* colonies were obtained by culturing them on mannitol salt agar and blood agar (Figure 1).

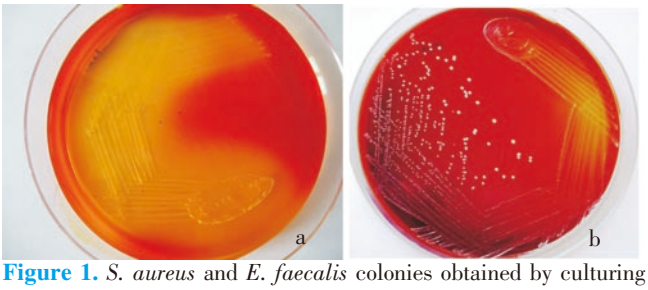

Figure 1. *S. aureus* and *E. faecalis* colonies obtained by culturing them on mannitol salt agar and blood agar.
a) *S. aureus* colonies; b) *E. faecalis* colonies.

Ethnobotanical information of 30 plants from aborigines along with their modalities in use is given in Table 1. Leaves

Table 1
Ethnomedicinal uses of plants used.

Sl. No	Plant name	Family	Local name	Parts used	Ethnomedicinal uses
1	*Aegle marmelos* L. Corr.	Rutaceae	Bela	Leaf	It is used in constipation, dysentery and diarrhoea. Leaves are used for treating diabetes, jaundice, cholera, asthma and ophthalmia.
2	*Anthocephalus cadamba* (Roxb.) Miq.	Rubiaceae	Kadamba	Leaf	Its bark is used in treatment of urinary infections and biliousness. It is used for diarrhoea, fever, inflammation, haemoptysis, cough, vomiting, wounds and ulcers.
3	*Argyreia speciosa* L.f.	Convolvulaceae	Brudha daraka	Leaf	Warm aqueous extract of A. cadamba leaves have been used to alleviate the wound healing and cuts.
4	*Aspidopterys tomentosa* (Blume) A. Juss	Malpighiaceae	Alatilaha	Root	Roots boiled in til (Sesamum indicum) oil is applied locally for treating eczema and itches.
5	*Azadirachta indica* L.Adelb	Meliaceae	Neem	Leaf	It is used as vermifuge and antiseptic as it is antibacterial and antiviral in action (chicken pox). It is used in the treatment of acne.
6	*Bacopa monnieri* L.Pennell	Scrophulariaceae	Brahmhi	Leaf	It helps protect the stomach from ulcer formation. It is useful in diarrhea and fevers, asthma and hoarseness.
7	*Butea monosperma* Lam. Taub	Fabaceae	Palasa	Leaf	It is useful diarrhoea, urine infections, leprosy, ulcers, tumours and skin diseases.
8	*Calotropis procera* (Aiton) W.T.Aiton	Asclepiadaceae	Arakha	Leaf	The powdered root controls asthma, bronchitis and antihelminthic. Its root-bark is used as a treatment for elephantiasis, leprosy, and in eczema. Leaves are useful intermittent fevers. Flowers are useful in asthma, catarrh, inflammations.
9	*Camellia sinensis* L. Kuntze.	Theaceae	Chai	Leaf	It possesses antibacterial, antiseptic, asthma. It is helpful in skin disorders
10	*Cassia fistula* L.	Caesalpiniaceae	Sunari	Leaf	It is useful in skin diseases, burning sensations and syphilis. It is useful in boils, leprosy, ringworm affection. It is useful in skin diseases, burning sensation, dry cough, bronchitis, dysentery and inflammations.
11	*Catharanthusroseus* L. G. Don	Apocyanaceae	Sadabihari	Leaf	It is used in case of nosebleed, bleeding gums, mouth ulcers and sore throats. It is also used internally for loss cystitis, gastritis and enteritis, diarrhea.
12	*Cissus quadrangularis* L.	Vitaceae	Hadajoda	Leaf	It is useful in eye and ear diseases and colic, leprosy, ulcers, tumours and skin diseases.
13	*Cleistanthus collinus* Hook.f. ex Planch.	Euphorbiaceae	Karla	Leaf	It is used as an anti-septic and against diarrhoea, amenorrhea.
14	*Dalbergia paniculata* (Roxb)	Fabacae	Sirisa	Leaf	In postnatal complaints, stem bark extract is administered with a pinch of pepper powder daily once for one week. for treating baldness and dandruff paste of dry system bark powder mixed with neem oil is applied
15	*Diospyrous melanoxylon* (Roxb)	Ebenaceae	Kendu	Leaf, bark	Used in urinary tract infection and skin trouble. Decoction of the bark is used in diarrhea and dyspsia.
16	*Elephantopusscaber* L.	Asteraceae	Mayurachulia	Leaf	Roots and leaves are reported for diarrhoea, dysentery, swellings and stomach pain. Powdered with pepper, it is applied for tooth-ache. Leaves are used in applications for eczema and ulcers.
17	*Ficus glomerata* Roxb	Moraceae	Dumer	Leaf	Leaves decoction are used against dysentery, diabetes, stomachache piles and diarrhoea.
18	*Glycyrrhiza glabra* L.	Fabaceae	Yasthimadhu	Leaf	It is useful in cough, bronchitis, ulcer, fever, hoarseness of voice, skin diseases, eye diseases, pharyngitis; also applied on cuts and wounds.
19	*Holarrhena antidysenterica* L Wall.	Apocyanaceae	Kutaja	Leaf, Bark	It is used for diarrhoea and skin diseases. The bark paste is mixed with cow urine and applied in affected skin parts. In treatment of urinary troubles, the bark is given with cow milk. The bark is used in chest affections and it is a well known herb for amoebic dysentery.
20	*Lantana camara* L	Verbenaceae	Nagaoiri	Leaf	Influenza, cough, mumps, incessant high fever, malaria, cervical lymph node tuberculosis, dermatitis, eczema, pruritus

Table 1, continued

Sl. No	Plant name	Family	Local name	Parts used	Ethnomedicinal uses
21	*Moringa oleifera* Lam.	Moringaceae	Sajana	Leaf	It acts as potent antitubercular and used to cure liver and is useful in diarrhoea, fever, inflammations, amenorrhea, dysmenorrheal, cough, and cold and eye diseases.
22	*Oroxylum indicum* L.Kurz	Bignoniaceae	Phaphen	Leaf, bark	Scabies, leprosy, diarrhoea, pyorrhea. During measles and swelling of body, a small piece of bark is rubbed in stone with water and applied over the body and a spoon full is given orally to arrest further growth.
23	*Pterocarpus santalinus* L. f.	Fabaceae	Raktachandan	Leaf, bark	It is used as an anti-septic, wound healing agent and anti-acne treatment. A decoction of fruit is used as an chronic dysentery.
24	*Salvodora persica* Wall	Salvadoraceae	Meswak	Bark	Leaves are useful in asthma, bronchitis, cough, painful tumors, verminosis. Shoots and leaves are used in treatment of cough and bronchitis. Tender twigs are used as toothbrush.
25	*Tectona grandis* L.	Lamiaceae	Teak	Bark	It is used as an anti-septic, wound healing agent and anti-acne treatment
26	*Terminalia alata* L.	Combretaceae	Sahaj	Leaf	For epilepsy, diarrhoea, dysentery aliquots of 20-30 mL of bark is given daily for a month or till symptoms disappear.
27	*Terminalia arjuna* L.	Combretaceae	Arjuna	Leaf, bark	The leave extracts inhibits skin diseases and urinary infection. It is used as expectorant. It acts against skin aliments including acne.
28	*Withania somnifera* L. Dunal	Solanaceae	Ashwagandha	Leaf	It has been used in diseases such as rheumatism, leprosy and arthritis.
29	*Woodfordia fruticosa* (L) Kurz.	Lythraceae	Dhatuki	Leaf	Used burning sensation, haemoptisis and liver disorder
30	*Vitex negundo* L.	Verbrenaceae	Nirgundi	Leaf	The dried fruit is vermifuge and is also used in the treatment of colds, coughs, diarrhoea, dysentery and acne treatment.

and/or barks of plants were used for obtaining aqueous and ethanolic extracts and those were concentrated by evaporating the solvents at 40 ℃ in a rotary evaporator.

Out of 708 positive clinical samples obtained over a period of 6 months (November 2009-April 2010) 391 were hospital-acquired (HA) isolates and 317 were community-acquired (CA) isolates. Of 391 HA isolates 293 isolates were *S. aureus* and 98 isolates were *E. faecalis*, and of 317 CA isolates 258 were *S. aureus* isolates and 59 strains were *E. faecalis* (Table 2). Individual types, Methicillin sensitive *S. aureus* (MSSA), Methicillin resistant *S. aureus* (MRSA), Vancomycin resistant *S. aureus* (VRSA), among *S. aureus*, and similarly, Vancomycin sensitive *E. faecalis* (VSE) and Vancomycin resistant *E. faecalis* (VRE) strains of *E. faecalis* were isolated (Table 2). Furthermore, MRSA occurred around 21.13% to 23.52% in all clinical samples; MSSA was more in CA than HA isolates at 27.76% and 17.39%, respectively; both VRSA and VSSA were prevalent in moderate values, 13% to 20%, approximately, in CA and HA samples; VRE and VSE were found in the range of 8% to 13% in both CA and HA samples (Table 2).

Antibiotics profiles of MRSA, MSSA and VRSA strains from both CA and HA isolates along with the reference drug sensitive strain MTCC 7443 *S. aureus*, against 17 antibiotics of 9 groups were recorded (Table 3). Similarly, antibiotic sensitivity of VRE and VSE from CA and HA along with the standard drug sensitive strain MTTC 439 *E. faecalis*, against 17 antibiotics were recorded (Table 3). It is discernable that all *S. aureus* strains were resistant to amikacin, gentamicin, penicillin, cefpodoxime and erythromycin. All *E. faecalis* strains were resistant to erythromycin, but 3 strains were resistant amikacin, ceftriaxone and azithromycin; two strains were resistant to amoxyclav and ampicillin, at doses specified in Table 3. Further, MRSA from CA was specifically sensitive to teicoplanin, vancomycin, co-trimoxazole, chloramphenicol and linezolid; MRSA from HA was specifically sensitive to linezolid and moderately sensitive to teicoplanin and azithromycin; MSSA strains were sensitive to many antibiotics (Table 3). VRSA from HA was sensitive to linezolid, whereas VRSA from CA was resistant to all antibiotics. Similarly, VRE isolates were almost resistance to all antibiotics, except VRE isolate from CA was sensitive to linezolid (Table 3).

Table 2

Hospital acquired and community acquired accounts of 708 Gram-positive isolates in a span of 6 months.

Bacterium	Number of isolates, n (%)		
	CA	HA	Total
MRSA	67 (21.13)	92 (23.52)	159
MSSA	88 (27.76)	68 (17.39)	156
VRSA	44 (13.88)	56 (14.32)	100
VSSA	59 (18.61)	77 (19.69)	136
VRE	27 (8.51)	47 (12.02)	74
VSE	32 (10.09)	51 (13.04)	83
Total	317 (100)	391 (100)	708

HA: hospital acquired; CA: community acquired. MRSA: Methicillin resistant *S. aureus*; MSSA: Methicillin sensitive *S. aureus*; VRSA: Vancomycin resistant *S. aureus*; VSSA: Vancomycin sensitive *S. aureus*; VRE: Vancomycin resistant *E. faecalis*; VSE: Vancomycin sensitive *E. faecalis*.

Both cold aqueous and ethanolic extracts of 30 plants were individually tested against 12 strains of *S. aureus* and

Table 3

Antibiotic susceptibility results of the selected gram positive multidrug resistant organisms.

Bacterium	Amino-glycosides		Beta-lactams				Cephalosporins		Fluoroquinolone	Glycopeptides		Macrol-ides		Lincosamide	Sulfon-amide	Stand alone	
	Ac	Ge	Ak	Am	Ox	P	Ctr	Cf	Of	Tei	Va	E	Az	Cd	Cot	Ch	Lz
S.a. MTCC	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
MRSA (CA)	R	R	R	MS	R	R	R	R	R	S	S	R	R	R	S	S	S
MRSA (HA)	R	R	R	R	R	R	R	R	R	MS	R	R	MS	R	R	R	S
MSSA (CA)	R	R	S	S	S	R	S	R	S	S	S	R	MS	S	R	R	S
MSSA (HA)	R	R	R	S	S	R	R	R	MS	S	S	R	S	R	R	R	S
VRSA (CA)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
VRSA (HA)	R	R	MS	R	R	R	R	R	MS	R	R	R	R	R	R	R	S
E.f. MTCC	S	S	S	S	nr	nr	S	S	S	S	S	S	S	S	S	S	S
VRE (CA)	R	MS	R	R	nr	nr	R	R	R	R	R	R	R	R	R	R	S
VRE (HA)	R	R	R	R	nr	nr	R	R	R	R	R	R	R	R	R	R	R
VSE (CA)	R	S	S	MS	nr	nr	S	S	S	S	S	R	S	S	S	S	S
VSE (HA)	S	S	S	R	nr	nr	R	R	S	S	S	R	R	S	S	S	S

Note: 'R'- Resistant; 'S'- Sensitive; 'MS'- moderately sensitive; 'nr'- not recommended. *S.a.*: *S. aureus*; *E.f.*: *E. faecalis*; MTCC: Microbial Type Culture Collections. Antibiotics (μg/disc): Ac: amikacin 30; Ak: amoxyclav 30; Am: ampicillin 10; Az: azithromycin 15; Cd: clindamycin 2; Cf: cefpodoxime 10; Ch: chloramphenicol 30; Cot: co-trimoxazole 25; Ctr: ceftriaxone 30; E: erythromycin 15; Ge: gentamicin 10; Lz: linezolid 30; Of: ofloxacin 5; Ox: oxacillin 1; P: penicillin 10; Tei: teicoplanin 30; Va: vancomycin 30.

Table 4

Result of screening of pathogenic bacteria by the agar-cup method with extracts of 30 medicinal plants given as zone of inhibition (mm).

Plants	Bacteria											
	S.a. MTCC	MRSA CA	MRSA HA	MSSA CA	MSSA HA	VRSA CA	VRSA HA	*E.f.* MTCC	VRE CA	VRE HA	VSE CA	VSE HA
1	17 (24)	17 (19)	- (15)	22 (19)	22 (20)	15 (13)	17 (14)	19 (23)	17 (20)	18 (15)	13 (19)	- (17)
2	17 (19)	15 (15)	- (18)	18 (23)	15 (26)	17 (19)	16 (19)	18 (19)	15 (19)	18 (26)	18 (21)	15 (18)
3	19 (26)	18 (22)	16 (20)	15 (19)	- (16)	- (16)	- (-)	18 (20)	15 (18)	- (15)	13 (17)	13 (22)
4	23 (24)	19 (20)	22 (24)	24 (20)	23 (22)	19 (23)	20 (19)	19 (21)	19 (18)	22 (19)	17 (19)	- (15)
5	19 (15)	15 (13)	12 (16)	16 (19)	17 (26)	- (17)	14 (22)	15 (18)	15 (20)	- (21)	- (20)	- (13)
6	16 (23)	16 (20)	13 (20)	17 (21)	- (22)	- (21)	- (18)	18 (20)	19 (18)	- (19)	15 (19)	- (18)
7	- (17)	- (17)	- (-)	- (18)	- (12)	- (19)	- (14)	15 (19)	- (15)	12 (16)	- (19)	15 (22)
8	- (19)	- (-)	- (14)	13 (17)	18 (22)	- (15)	- (-)	- (15)	- (17)	- (18)	14 (19)	17 (22)
9	15 (18)	12 (15)	14 (16)	18 (20)	15 (27)	15 (19)	14 (18)	12 (17)	17 (15)	15 (19)	19 (20)	12 (18)
10	- (19)	- (16)	- (-)	- (16)	- (-)	- (16)	- (-)	- (16)	- (19)	- (14)	- (17)	- (22)
11	16 (23)	17 (23)	23 (25)	19 (26)	22 (25)	21 (26)	19 (21)	23 (27)	20 (24)	17 (21)	17 (20)	15 (26)
12	11 (14)	- (16)	14 (18)	18 (20)	14 (20)	- (15)	- (19)	- (14)	- (-)	11 (17)	- (19)	- (22)
13	15 (19)	16 (15)	- (17)	12 (16)	17 (18)	- (16)	12 (19)	- (17)	- (15)	- (16)	15 (20)	- (24)
14	- (12)	12 (15)	- (-)	- (18)	- (15)	- (15)	- (-)	- (-)	15 (18)	- (-)	- (15)	- (19)
15	24 (27)	21 (23)	23 (24)	24 (25)	21 (24)	24 (25)	23 (25)	25 (26)	23 (24)	24 (23)	24 (27)	23 (26)
16	- (15)	- (15)	- (-)	- (17)	15 (22)	- (15)	- (19)	15 (19)	15 (20)	- (16)	15 (22)	15 (22)
17	15 (19)	12 (17)	- (17)	14 (19)	- (22)	- (18)	- (15)	15 (20)	12 (22)	- (18)	13 (20)	- (25)
18	18 (21)	15 (17)	12 (17)	15 (19)	- (24)	16 (20)	12 (21)	20 (25)	19 (21)	16 (19)	19 (26)	18 (22)
19	20 (26)	17 (19)	14 (17)	14 (20)	- (22)	16 (19)	15 (22)	19 (22)	15 (20)	- (19)	16 (24)	17 (25)
20	21 (22)	23 (25)	24 (26)	24 (27)	25 (27)	22 (24)	25 (26)	21 (23)	24 (26)	22 (24)	24 (27)	23 (24)
21	16 (20)	12 (19)	- (20)	- (18)	- (20)	- (-)	- (14)	17 (20)	15 (17)	- (21)	15 (19)	14 (22)
22	28 (24)	33 (24)	26 (19)	36 (18)	33 (22)	26 (19)	24 (17)	15 (20)	18 (22)	15 (20)	18 (22)	- (19)
23	24 (27)	21 (23)	23 (26)	24 (27)	22 (24)	24 (17)	23 (25)	25 (26)	21 (24)	24 (23)	24 (25)	23 (26)
24	15 (19)	15 (18)	15 (19)	18 (22)	16 (27)	- (19)	15 (20)	- (17)	- (20)	- (18)	12 (17)	- (24)
25	20 (22)	23 (25)	23 (24)	21 (25)	20 (22)	24 (26)	23 (26)	22 (24)	21 (25)	21 (23)	20 (22)	21 (22)
26	- (-)	- (14)	- (-)	- (-)	- (-)	- (18)	- (14)	- (18)	- (17)	- (15)	- (20)	- (22)
27	- (18)	- (20)	- (17)	- (20)	- (22)	- (17)	- (18)	- (17)	- (17)	- (15)	- (19)	15 (22)
28	16 (19)	17 (19)	14 (19)	14 (17)	- (20)	- (18)	14 (18)	- (17)	- (15)	- (-)	- (16)	- (23)
29	22 (25)	21 (25)	22 (26)	20 (24)	21 (22)	25 (26)	22 (26)	24 (27)	21 (25)	21 (23)	24 (27)	20 (22)
30	- (16)	- (17)	- (19)	12 (21)	15 (22)	- (14)	- (17)	16 (19)	16 (23)	14 (19)	15 (19)	- (27)

Numbers 1 to 30 are serial numbers of plants given in Table 1; abbreviations of bacteria are given in Table 2. Values outside the parentheses are measurements of zones of inhibition due to water-extracts and values in parentheses are those due to ethanol-extracts. "_" sign denotes no activity.

Table 5
Preliminary phytochemical analyses of aqueous and ethanolic extracts of the plants.

Sl. No	Plants	Alkaloids	Glycosides	Terpenoids	Reducing sugars	Saponins	Tannins	Flavonoids	Steroids
1	*A. marmelos*	− (+)	+ (−)	+ (+)	+ (+)	+ (−)	− (+)	+ (+)	+ (+)
2	*A. cadamba*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
3	*A. speciosa*	− (+)	− (+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)	− (+)
4	*A. indica*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
5	*A. tomentosa*	− (+)	− (−)	− (−)	+ (+)	+ (+)	− (+)	+(+)	− (−)
6	*B. monnieri*	+ (+)	+ (+)	− (+)	− (+)	− (+)	+ (+)	+ (+)	− (+)
7	*B. monosperma*	+ (+)	+ (−)	+ (+)	− (+)	+ (−)	− (+)	+ (+)	+ (−)
8	*C. procera*	− (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)
9	*C. sinensis*	− (+)	− (−)	− (+)	+ (+)	− (−)	− (+)	− (−)	− (+)
10	*C. fistula*	+ (+)	− (+)	− (+)	+ (−)	+ (+)	+ (+)	+ (+)	− (−)
11	*C. roseus*	+ (+)	− (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (−)
12	*C. quadrangularis*	+ (+)	− (−)	− (+)	− (−)	− (+)	− (+)	− (+)	− (+)
13	*C. collinus*	− (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)	− (+)
14	*D. paniculata*	− (+)	+ (+)	− (−)	− (+)	− (+)	+ (−)	− (+)	− (+)
15	*D. melanoxylon*	+ (+)	− (+)	− (−)	− (−)	+ (+)	−(+)	− (+)	+ (+)
16	*E. scaber*	− (+)	− (+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)	− (+)
17	*F. glomerata*	+ (+)	− (+)	− (+)	+ (−)	+ (+)	+ (+)	+ (+)	− (−)
18	*G. glabra*	+ (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
19	*H. antidysenterica*	+ (+)	+ (+)	+ (+)	+ (+)	− (+)	− (−)	− (+)	+ (+)
20	*L. camara*	− (+)	+ (+)	− (+)	− (+)	− (+)	− (+)	+ (+)	− (+)
21	*M. oleifera*	+ (−)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
22	*O. indicum*	− (+)	+ (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	− (+)
23	*P. santalinus*	− (−)	+ (+)	− (−)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)
24	*S. persica*	+ (+)	+ (+)	− (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)
25	*T. grandis*	− (−)	+ (+)	− (−)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)
26	*T. alata*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
27	*T. arjuna*	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)	+ (+)
28	*W. somnifera*	+ (+)	+ (+)	+ (+)	− (+)	+ (+)	+ (+)	+ (+)	+ (+)
29	*W. fruticosa*	+ (+)	− (+)	− (+)	− (+)	−(+)	−(+)	+ (+)	− (+)
30	*V. negundo*	+ (+)	− (+)	+ (+)	+ (−)	− (+)	+ (+)	+ (+)	+ (+)

Note: "+" sign denotes presence, and "−"sign denotes absence of the compound in a plant; signs outside denotes about a phyto-chemical in water extract, and sign in parentheses denotes in ethanolic extract.

E. faecalis: two MRSA, two MSSA, and two VRSA along with standard strains; similarly two VRE and two VSE along with the standard strain. Both aqueous and ethanolic extracts plants, *Diospyrous melanoxylon* (*D. melanoxylon*), *Woodfordia fruticosa* (*W. fruticosa*), *Oroxylum indicum* (*O. indicum*), *Dalbergia paniculata* and *Lantana camara* (*L. camara*) had the most *in vitro* controlling capacity against the MDR *S. aureus* and *E. faecalis*. Again, extracts of *Holarrhena antidysenterica*, *Aspidopterys tomentosa* and *Argyreia speciosa* had moderate antibacterial activities. Ethanolic extracts of *Cassia fistula* had moderate antibacterial activities over all the 11 strains except VSE isolated from hospital whereas its corresponding aqueous extracts did not have any effect on any of the strains. It was evident from the study that most of the ethanolic extracts of all the plants used possessed some amount antibacterial activity, which indicates that they have better antibacterial activities than the corresponding aqueous extracts (Table 4).

Preliminary phytochemical analyses were done for both extracts of all the 30 plants. Ethanolic extracts of *L. camara*, *O. indicum* and *W. fruticosa* contained all the phytochemicals (alkaloids, glycosides, terpenoids, reducing sugars, saponins, tannins, flavonoids and steroids), which could be attributed to the recorded significant antibacterial activity. Certain extracts such as the aqueous extract of *D. melanoxylon* did not contain flavonoids, but its alcoholic extract contained flavonoids. Presence of such phyto-compounds in individual extracts cumulatively redounds to the antibacterial activities of plants. The results of phytochemical analysis of all plants are recorded in Table 5.

4. Discussion

As it is known, long term hospitalization causes increase of susceptibility of a patient to the MRSA infection[28], particularly at surgical sites and urinary tracts. It was also reported that 51.5% of infected MRSA patients had already been infected at their time of admission to hospitals, which cause an introduction of new MRSA strains to hospitals from community, in reality[29]. Further, in England and Wales, less than 2% of *S. aureus* strains were methicillin-resistant in 1990, but by 2002 an eyebrow-raising 42% of *S. aureus* strains were methicillin-resistant; approximately 300 000 cases of nosocomial MRSA infections were estimated each year leading to 5 000 deaths[30]. Vancomycin has always been the choice of drug in the cases of MRSA infections[31], but in our study fully resistant or partially resistant

vancomycin intermediate *S. aureus* (VISA) and VRSA have been reported, which suggests that this antibiotic could be resistance to MRSA in a short. The development of VISA is suspected due to combination therapy of vancomycin with an aminoglycoside (gentamicin), for a synergistic action of the two antibiotics[32].

Enterococci are primarily opportunistic pathogens. Intensive use of broad spectrum antibiotics in hospitals could be responsible for the emergence of these pathogens and eventual nosocomial spreads[33]. The first report of VRE was reported in 1988[34]. Thereafter, VRE have spread rapidly all over the world. For example, from the year 1989 to 1993 the percentage of nosocomial infections due to VRE reported to the Centers for Disease Control and Prevention, USA increased from 0.3 to 7.9%[35]. Though the major problem in treatment of VRE infection arises in endocarditis, the urinary tract is the commonest site from where bacteraemia can occur. There are very few reports on isolation of VRE from India[36], though the epidemiology of nosocomial VRE bacteraemia has been quite extensively studied. Studies on problems posed by the VRE as pathogens in UTI are very few. Enterococci in mixed culture are very commonly isolated from urine samples. It is not always easy to assess the clinical significance of VRE in routine culture or to differentiate colonization from infection[37]. The present study was undertaken to look for vancomycin resistance in Enterococci obtained in significant numbers from various HA and CA samples, and to study the infection dynamics of this MDR pathogen.

Nosocomial acquisition and its subsequent colonization of VRE is an emerging international threat to public health, and it has been emphasized in the United States; colonization among non hospitalized persons has been also reported. In contrast, in European countries, colonization is frequently reported in persons outside the health-care settings[38]. An important factor associated with VRE demonstrated in the in European community that the avoparcin, a glycopeptide antimicrobial drug used for years at sub-therapeutic doses as a growth promoter in food-producing animals induce VRE; evidences suggested that food-borne VRE landed in human colonization, with whom no or limited vancomycin was used, earlier[39,40].

In a study with phytochemicals from Mysore, India, *W. fruticosa* was reported to have antibacterial activity against standard MTCC strains of GP pathogens *S. aureus* and *Streptococcus faecalis*, having zones of inhibition more than 21 mm, which was more than the zone of inhibition of positive controls, the antibiotic gentamicin. In the same study, the same plant showed a great deal of antibacterial activity against the other standard MTCC Gram-negative pathogens, particularly against *Salmonella paratyphi B*, *Shigella boydii* and *Sh. dysenteriae*[41]. Herein, different resistant patterns of clinical isolates of the two GP strains were recorded, along with the extract of *W. fruticosa* that registered a good *in vitro* controlling capacity on both pathogens. Phytocompounds in *L. camara* viz., pentacyclic triterpenes were found active against *S. aureus* and *Salmonella typhi*[42]. Also a number of furano-naphthoquinones have been shown to possess antimicrobial activity against GP bacteria and fungi[43]. A report from Bangladesh recorded the antibacterial activity of multi solvent extracts of *O. indicum* bark against various Gram-negative and GP bacteria. Particularly hexane, chloroform and carbon tetrachloride extracts showed significant activity against *Bacillus megaterium*, *S. paratyphi*, *Vibrio mimicus*, *V. parahaemolyticus*, *Pseudomonas aeruginosa*, *B. cereus*, *B. subtilis* and *E. coli*[44]. Another plant, *Celastrus paniculata* showed antibacterial activity against *Streptococcus pyogenes*, *B. subtilis*, *B. cereus*, *Corynebacterium diphtheriae*, *S. typhi*, *S. paratyphi* A and B, *E. coli*, *Pseudomonas*, *S. aureus*, *Klebsiella pneumoniae* and *Proteus vulgaris*. Again aqueous extract of *C. paniculata* seed had potent antibacterial activity against *B. cereus*, *K. pneumoniae*, *P. vulgaris*, *S. typhi*, *S. paratyphi* A, *E. coli*, *P. aeruginosa* and *S. aureus*[45].

S. aureus strains (MRSA and VRSA) have been found as the most widely prevailing pathogens in nosocomial settings, than in community. Plants, *L. camara*, *W. fruticosa*, *O. indicum* and *P. santalinus*, were proved to have notable *in vitro* control on MDR strains of both pathogens, particularly could be useful as complementary/ supplementary/ alternative therapeutic agents against GP pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work is a part of PhD thesis of DD in Microbiology of Utkal University; she is an INSPIRE Fellow (If10503/2011) of DST, Govt. of India, New Delhi. RNP is grateful to Council of Scientific & Industrial Research, New Delhi, for an award as 'Scientist'. This work supported by a Major Research Project on Botany[39-388/2010(SR)] titled 'Alternative drug search from ethnomedicinal plants of Odisha against multidrug resistant bacteria' awarded to RNP, by University Grants Commission, New Delhi. We are grateful to Dr. NK Debata, Microbiology Department, and Mr. Gopabandhu Kar, Managing Member, Sum Hospital (S'O'A University) for extended facilities.

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