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## Surveillance of multidrug resistant suppurative infection causing bacteria in hospitalized patients in an Indian tertiary care hospital

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### ABSTRACT

**Objective:** To examine antibiograms of a cohort of suppurative bacteria isolated from wound–swabs from hospitalized patients of all economic groups of a typical Indian teaching hospital. **Methods:** In surveillance, antibiotic resistance patterns of 10 species of suppurative bacteria isolated from wound–swabs over a period of 24 months were recorded. Those were subjected to antibiotic sensitivity test, using 16 prescribed antibiotics of 5 different groups (3 aminoglycosides, 4 beta–lactams, 3 cephalosporins, 4 fluoroquinolones, and 2 stand–alone) in each 6–month interval of the study period. **Results:** Of 1 156 samples collected, 819 samples yielded pathogenic bacteria, of which, *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (*S. pyogenes*), *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterococcus faecalis* (*E. faecalis*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*), *Enterobacter aerogenes* (*E. aerogenes*), *Proteus mirabilis* (*P. mirabilis*) and *Proteus vulgaris* (*P. vulgaris*) were isolated in the order of predominance. Isolated bacterial strains were floridly multidrug resistant. Strains of *E. faecalis* and *S. aureus* were found resistant to vancomycin, one of the newly introduced antibiotics. **Conclusions:** Of these *S. aureus*, particularly the methicillin resistant strain predominates, followed by strains of *S. pyogenes* and *P. aeruginosa* that were in the higher proportions of multidrug resistance.

## 1. Introduction

Infections at burn and surgical wounds with Gram–positive (GP) and Gram–negative (GN) aerobic bacteria have become a constant source of consternation to clinicians and surgeons. In the last few decades, management of wounds and associated co–morbidity has been creating increasing levels of failures in their control, because of the emergence of multidrug resistant (MDR) bacteria, escalating to other body parts as blood stream infections (BSIs). Moreover, the decaying part of a burn–wound picks up opportunistic infections including fungi even, widening the opportunity for super–infection by several bacteria, both from

community/hospital settings originally unrelated to skin; eventually, this leads to bacteremia in several vital organs idiosyncratic to type and the extent of drug resistance of the infecting bacterium[1]. Bacteria, *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecalis* (*E. faecalis*) and *Streptococcus pyogenes* (*S. pyogenes*), as GPs, and *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella* sp., *Escherichia coli* (*E. coli*), *Proteus* sp., *Haemophilus* sp. and *Moraxella* sp., among GN aerobic bacteria, as well as *Bacteroides* sp. and *Fusobacterium* sp., as anaerobic ones, are found mainly causing suppurations at wound sites, which lead to cystitis, otitis, boils, mastitis, phlebitis, meningitis, pneumonia, osteomyelitis, endocarditis urinary tract infection (UTI), and a few more[2]. Among opportunistic GNs, *P. aeruginosa* especially is the ill–famed notorious pathogen linked to doggedly intractable burn injuries[3], promoting urinary tract infections (UTIs) even, leading to renal failure or/and endocarditis leading to heart failure, due to suppurative bacteria with portal entry as BSI[4]. Indeed, extrication from

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the pandemonium of burn injury is a staggering victory, and its management looms large to the clinician due to multiple infections by MDR bacteria. Specifically, infected surgical wounds cause suppurative skin reactions, bacterial fluid lesions and subcutaneous nodules leading to metastasis, when not properly addressed. An unattended/ unaddressed wound–site is the most vulnerable point of entry of the marauding pathogen that may cause bacteremia latter on, because of its multidrug resistance. Indeed, certain GN bacteria (*P. aeruginosa*, *Acinetobacter baumannii* (*A. baumannii*), and *K. pneumoniae*) have emerged with versatile strains, resistance to all antibiotics of the present time and such strains are informally known as pandrug resistant (PDR) bacterial<sup>[5,6]</sup>.

*S. aureus*, once known as a harmless commensal of nasal nares and soft tissues of the body, has become resistant to oxacillin/methicillin (penicillin–derivatives), and is known as, methicillin resistant *S. aureus* (MRSA). In an earlier study, IMS and Sum Hospital, Bhubaneswar, had reported resistance of MRSA to 23 antibiotics used at the present time<sup>[7,8]</sup>, confirming it as the ‘superbug in health domain’, in this zone too. Intrinsically, MRSA has become the most common suppurative pathogen worldwide today, due to the armamentarium of multiple resistances, often creating an intractable situation in intensive and critical care units<sup>[7–9]</sup>. From the available literature, it could be spelled out that, *S. aureus* has several factors of potential virulence: (1) surface proteins promoting colonization in host tissues; (2) invasion–promoting proteins causing spread in host tissues; (3) surface factors inhibiting phagocytic engulfment by host cells of immune system, due to bacterial capsule and proteinase–A production; (4) inherent biochemical properties such as, catalase production; (5) immunological disguises such as, proteinase–A and coagulase productions by bacteria; (6) production of membrane degrading toxins, haemotoxin and leukotoxin that modify properties of eukaryotic cell membrane; (7) exotoxin production that damages host tissues by provoking symptoms of a disease<sup>[10]</sup>. Thus, the majority of diseases caused by *S. aureus* or MRSA and its other recent clonal nexuses are multifactorial. Human staphylococcal infections are more frequent, but they usually remain at the local entry spot because of limited activity of the host defense system at the injury spot. A hair follicle, needle–stick injury or a surgical wound itself would be common spots of portal entry of a pathogen. *Staphylococcal pneumonia* (*S. pneumonia*) is the frequent complication of pneumonia causing swelling, accumulation of pus and additional necrosis of lung–tissue in a more serious situation. Moreover, the inflamed area due to *S. aureus* is often a fibrin clot of bacteria and dead leukocytes, as a pus–filled boil or an abscess<sup>[11]</sup>. More serious infections by *S. aureus* of skin are furuncles or impetigo, and the localized bone infection is osteomyelitis; but BSI of *S. aureus* leads to septicemia and bacteremia attacking lungs, kidney, heart, skeletal muscles and

meninges, at least in old and immunocompromised patients, eventually causing utmost morbidity and significant mortality<sup>[1]</sup>. Other suppurative GP bacteria are *Streptococcus* and *Enterococcus*, which have developed parallel resistance to many antibiotics of the time<sup>[12]</sup>. More often than not, multiple bacterial infections are commonplace at injury sites.

In face of accumulation of a vast majority of literature on MDR bacteria, it has become a matter of compulsion to conduct a regional surveillance on this exasperating class of pathogens, causing morbidity and mortality from general and surgical wound sites mainly<sup>[13]</sup>. This study records a gamut of antibiograms using 16 antibiotics of 5 groups of the time, with three GP and seven GN bacteria isolated from wound–swabs of hospitalized patients of a typical Indian tertiary care hospital, a systematic study never reported before. This study should strengthen the epidemiological database of this vast subtropical country and other workers for a comparison as well as, in setting the stringent control of infections in a hospital. It is anticipated that this work would also benefit the pharmacy–world for further strategies in the crusade of the control of MDR bacteria, as wound infections give way to BSI and many more comorbidities. The infection dynamics of each bacterium studied here is so vast in literature that they would fill a book or two, when attempts would be made to describe their consternation in clinical management. This work examines antibiograms of a cohort of suppurative bacteria isolated from wound–swabs during 24 months from wards and cabins of the hospital, which treats patients of all economic groups, from slum dwellers and rural rustics to elite mass.

## 2. Materials and methods

### 2.1. Isolation and identification of bacteria

Strains of three GP, *E. faecalis*, *S. aureus*, *S. pyogenes*, and seven GN *A. baumannii*, *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris*, and *P. aeruginosa* were isolated from the in–house patients during the study period. The isolated GP and GN bacteria were identified basing upon their colony morphology and results obtained from standard biochemical test, as done previously<sup>[7,14,15]</sup>. Microbial Type Culture Collection (MTCC) strain of each GP or GN bacterium was used as the reference control in each biochemical test.

### 2.2. Antibiotic susceptibility test

All bacterial strains including the standard MTCC strains of each bacterium were subjected to antibiotic sensitivity tests by the Kirby–Bauer’s method/ disc diffusion method, using a 4 mm thick Mueller–Hinton (MH) agar (HiMedia, Mumbai) medium<sup>[16]</sup>. An aliquot of 0.1 mL of 0.5 McFarland

equivalents, approximately from an exponentially growing culture was spread on agar for the development of lawn of a strain of a bacterium at 37°C in a BOD incubator (Remi CIM–12S). Further, on the lawn–agar of each plate, 8 high potency antibiotic discs (HiMedia) of 16 prescribed antibiotics of 5 different groups were placed, individually at equal distances from one another. Plates were incubated for 18 h at 37°C and were examined for size–measurements of zones of inhibition around each disc, following the standard antibiotic susceptibility test chart of Clinical Laboratory Standard Institute (CLSI) guidelines<sup>[7]</sup>.

### 3. Results

From hospitalized patients of wards and cabins of IMS and Sum Hospital, 1 156 wound–swabs samples yielded 819 strains of pathogenic bacteria belonging to 10 species (three GP and seven GN bacteria) during the span of 24 months (April 2011– March 2013). All strains (*E. faecalis*, *S. aureus*, *S. pyogenes*, *A. baumannii*, *E. coli*, *E. aerogenes*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris* and *P. aeruginosa*) were identified by standard biochemical tests and were maintained as axenic cultures in suitable media. In total, there were 52 strains of *E. faecalis*, 251 strains of *S. aureus*, 210 strains of *S. pyogenes*, 48 strains of *A. baumannii*, 39

**Table 1**

Bacteria isolated from wound–swabs of the in–house wards patients.

Bacteria	April – September 2011	October 2011– March 2012	April–September 2012	September 2012–March 2013	Total
<i>E. faecalis</i> *	18	7	17	10	52
<i>S. aureus</i> *	61	67	76	47	251
<i>S. pyogenes</i> *	65	59	57	29	210
<i>A. baumannii</i>	12	16	11	9	48
<i>E. aerogenes</i>	11	6	16	6	39
<i>E. coli</i>	16	18	17	11	62
<i>K. pneumoniae</i>	16	11	19	7	53
<i>P. mirabilis</i>	7	6	7	4	24
<i>P. vulgaris</i>	7	4	9	1	21
<i>P. aeruginosa</i>	16	21	14	8	59
Grand total					
(percentage values)	229 (27.96)	215 (26.25)	243 (29.67)	132 (16.11)	819

Note: Total samples collected, 1156; \* Gram–positives.

**Table 2**

Media used for isolation and maintenance pathogenic bacteria from urine samples and their colony characteristics.

Bacterium	Standard MTCC strain number	Media used	Colony characteristics
<i>E. faecalis</i>	439	Blood agar	Grey coloured, round, gamma hemolytic colonies
<i>S. aureus</i>	7443	Blood agar	Medium to large, smooth, entire, slightly raised, creamy yellow, with green/beta–hemolytic colonies
		Nutrient agar	As above without hemolytic activity
<i>S. pyogenes</i>	1928	Blood agar	Beta–haemolytic colonies
<i>A. baumannii</i>	1425	Nutrient agar	Colourless smooth, opaque, raised and pinpoint
		MacConkey agar	Colourless smooth, opaque, raised, NLF
		CLED agar	Blue coloured opaque raised NLF
<i>E. aerogenes</i>	2990	Blood agar	White convex with gamma hemolysis
		MacConkey agar	LF, mucoid
<i>E. coli</i>	443	Nutrient agar	Flat dry, irregular
		MacConkey agar	LF, flat dry pink, irregular
		EMB agar	Purple coloured, flat dry, irregular colonies, with metallic green colour.
		Blood agar	Swarms on blood agar with beta–hemolysis
		CLED agar	Translucent blue
<i>K. pneumoniae</i>	4031	MacConkey agar	LF, pink, mucoid
		CLED Agar	Yellow mucoid
<i>P. mirabilis</i>	NA	MacConkey agar	LLF light pink after 67 h
		Blood agar	Swarms on blood agar with beta–hemolysis
		CLED agar	Translucent blue
<i>P. vulgaris</i>	1771	Blood agar	Swarms on blood agar with beta–hemolysis
		CLED agar	Translucent blue
<i>P. aeruginosa</i>	1688	Nutrient agar	Large, irregular opaque with bluish green pigment

Note: CLED, Cysteine lactose electrolyte deficient; EMB, Eosin–methylene blue, Lactose fermenting; NLF, Non–lactose fermenting; NA, not available.

strains of *E. aerogenes*, 62 strains of *E. coli*, 53 strains of *K. pneumoniae*, 24 strains of *P. mirabilis*, 21 strains of *P. vulgaris* and 59 strains of *P. aeruginosa*. Thus, *S. aureus* was the maximally isolated suppurative–infection–causing bacterium, followed by *S. pyogenes*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *E. faecalis*, *A. baumannii*, *E. aerogenes*, *P. mirabilis* and *P. vulgaris* (Table 1). During the last 6–month period, minimum numbers of pathogens were isolated. GP bacteria as medium to large, smooth, entire, slightly raised, creamy yellow, green–coloured beta–haemolytic colonies on blood agar, found positive to catalase and coagulase tests were confirmed as *S. aureus*. Similarly, characteristics the rest other GP and GN bacteria are presented (Tables 2 and 3).

All isolated bacterial strains were subjected to antibiotic sensitivity tests with all antibiotics used, in each 6–month period. Three aminoglycoside antibiotics ( $\mu$  g/disc), amikacin–30, gentamicin–10 and tobramycin–10 were moderately resistant to ten species of pathogens used, in ranges, 27% to 76% of 52 strains of *E. faecalis*, 31% to 79% of 251 strains of *S. aureus*, 51% to 76% of 210 strains of *S. pyogenes*, 25% to 71% of 48 strains of *A. baumannii*, 36% to 78% of 39 strains of *E. aerogenes*, 61% to 81% of 62 strains

of *E. coli*, 66% to 79% of 53 strains of *K. pneumoniae*, 66% to 89% of 24 strains of *P. mirabilis*, 61% to 71% of 21 strains of *P. vulgaris*, 66% to 86% of 59 strains of *P. aeruginosa*. Among these three antibiotics, amikacin was the most resistant antibiotic to these pathogens (Table 4). Mean percent values of resistant strains of individual bacteria are discernible (Table 4).

Similarly, percentages of resistance patterns of 3 GP bacteria with four antibiotics of the beta–lactam group are detailed (Table 5); resistance patterns were in ranges: 52 to 74% of 52 strains of *E. faecalis*, 61 to 82% of 251 strains of *S. aureus*, and 47 to 76% of 210 strains of *S. pyogenes*. Likewise, GN bacteria were tested for three beta–lactams only with resistance patterns as given: 47% to 77% of 48 strains of *A. baumannii*, 52% to 81% of 39 strains of *E. aerogenes*, 51% to 89% of 62 strains of *E. coli*, 54% to 83% of 53 strains of *K. pneumoniae*, 22% to 43% of 24 strains of *P. mirabilis*, 34% to 48% of 21 strains of *P. vulgaris*, 41% to 75% of 59 strains of *P. aeruginosa*. For GN bacteria, antibiotics were resistant in the order: ampicillin > piperacillin/ tazobactam > amoxycylav. But with GP bacteria such an order would be: ampicillin > piperacillin/ tazobactam > oxacillin > amoxycylav (Table 5). Mean percent

**Table 3**

Biochemical identifications of isolated Gram–positive and Gram–negative bacteria.

Bacteria	Catalase	Oxidase	Coagulase	Indole	MR	VP	Citrate	Urease	TSI	Nitrate	Bacitracin	Bile esculin
<i>E. faecalis</i>	–	Nd	–	Nd	Nd	Nd	Nd	Nd	Nd	Nd	–	+
<i>S. aureus</i>	+	Nd	+	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
<i>S. pyogenes</i>	+	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	+	Nd
<i>A. baumannii</i>	+	–	Nd	–	–	+	+	V	nd	–	Nd	Nd
<i>E. aerogenes</i>	+	–	Nd	–	–	+	+	–	A/A	+	Nd	Nd
<i>E. coli</i>	+	–	Nd	+	+	–	–	–	A/AG	+	Nd	Nd
<i>K. pneumoniae</i>	+	–	Nd	–	–	+	+	+	A/AG	+	Nd	Nd
<i>P. mirabilis</i>	+	–	Nd	–	+	–	+	+	K/A H <sub>2</sub> S	+	Nd	Nd
<i>P. vulgaris</i>	+	–	Nd	+	+	–	+	+	K/A H <sub>2</sub> S	+	Nd	Nd
<i>P. aeruginosa</i>	+	+	Nd	–	–	–	+	+	Nd	+	Nd	Nd

Note: MR, methyl red test; VP, Voges–Proskauer test; TSI, triple sugar iron test; V, variable; A/A, acid in slant and butt; A/AG H<sub>2</sub>S, acid in slant and butt with H<sub>2</sub>S gas production; A/AG, acid in slant and butt with gas production; K/A H<sub>2</sub>S, alkali in slant and butt with H<sub>2</sub>S gas production. Nd, not done; +, positive; –, negative

**Table 4**

Percentage of resistance of all clinically isolated bacteria to three antibiotics of aminoglycoside group.

Bacterium	Aminoglycosides														
	Amikacin 30 $\mu$ g/disc					Gentamicin 10 $\mu$ g/disc					Tobramycin 10 $\mu$ g/disc				
	I	II	III	IV	Mean	I	II	III	IV	I	II	III	IV	Mean	
<i>E. faecalis</i>	56	67	76	64	65.75	47	56	59	54	27	34	41	39	35.25	
<i>S. aureus</i>	67	76	79	71	73.25	57	59	56	58	31	49	56	54	47.50	
<i>S. pyogenes</i>	62	67	76	75	70.00	64	69	73	74	51	55	62	65	58.35	
<i>A. baumannii</i>	67	68	71	63	67.25	54	61	63	65	25	31	36	38	31.50	
<i>E. aerogenes</i>	66	78	77	73	73.50	48	51	57	68	36	40	41	41	39.50	
<i>E. coli</i>	71	76	81	79	76.75	76	79	80	78	61	67	65	69	65.50	
<i>K. pneumoniae</i>	66	76	79	79	75.00	67	69	67	66	66	78	77	75	74.00	
<i>P. mirabilis</i>	67	69	78	74	72.00	89	67	66	69	87	71	76	75	77.25	
<i>P. vulgaris</i>	66	69	67	70	68.00	69	67	71	70	66	67	61	65	64.75	
<i>P. aeruginosa</i>	76	77	76	72	75.25	86	77	67	68	76	68	66	67	69.25	

Note: I, April – September 2011; II, October 2011 – March 2012; III, April 2012 – September 2012; IV, November 2012 – March 2013

values of resistant strains of individual bacteria are evident in the Table 5.

Further, resistance–percent values of suppurative–infection–causing bacteria to the cephalosporin group (cefepime, ceftazidime and ceftriaxone), in three 6–month phases were in ranges, 57% to 76% of 52 strains of *E. faecalis*, 51% to 76% of 251 strains of *S. aureus*, 51% to 85% of 210 strains of *S. pyogenes*, 45% to 64% of 48 strains of *A. baumannii*, 52% to 72% of 39 strains of *E. aerogenes*, 75% to 88% of 62 strains of *E. coli*, 54% to 74% of 53 strains of *K. pneumoniae*, 32% to 51% of 24 strains of *P. mirabilis*, 36% to 46% of 21 strains of *P. vulgaris*, 49% to 77% of 59 strains of *P. aeruginosa* (Table 6). All these three antibiotics were almost equally resistant to the isolated suppurative pathogens, confirming the consistence in the production of ESBL by majority of isolates.

Similarly, resistance–percent values of suppurative infection causing bacteria to antibiotics of the fluoroquinolone group (gatifloxacin, levofloxacin, ciprofloxacin and ofloxacin) in three 6–month phases were in ranges, 51% to 76% of 52 strains of *E. faecalis*, 68% to 89% of 251 strains of *S. aureus*, 44% to 65% of 210 strains of

*S. pyogenes*, 45% to 77% of 48 strains of *A. baumannii*, 47% to 81% of 39 strains of *E. aerogenes*, 60% to 87% of 62 strains of *E. coli*, 58% to 81% of 53 strains of *K. pneumoniae*, 25% to 49% of 24 strains of *P. mirabilis*, 25% to 45% of 21 strains of *P. vulgaris*, 54% to 81% of 59 strains of *P. aeruginosa* (Table 7). These antibiotics were resistant to all these pathogens in the order: ofloxacin > gatifloxacin > norfloxacin > levofloxacin; the later one was newly introduced.

Lastly, detailed percentage values of GP bacteria were: 26% to 32% of 52 strains of *E. faecalis*, 36% to 39% of 251 strains of *S. aureus* and 34% to 47% of 210 strains of *S. pyogenes* for linezolid. The resistance patterns of 2 GP bacteria to the stand–alone antibiotic, vancomycin were in ranges was in ranges, 15% to 19% of 52 strains of *E. faecalis*, and 11% to 17% of 251 strains of *S. aureus* (Table 8). The emergence of vancomycin resistant mutants is a matter of clinical concern. Of 819 bacterial isolates in this study, *S. aureus*, *S. pyogenes*, *P. aeruginosa*, *E. faecalis*, *K. pneumoniae*, *A. baumannii*, *E. aerogenes* and *Proteus* sp. were found in the order of predominance to antibiotic resistance. This study recorded that all of these pathogens isolated from clinical samples were resistant to all antibiotics individually used.

**Table 5**

Percentage of resistance of all clinically isolated bacteria to four antibiotics of  $\beta$ –lactam group.

Bacterium	Beta–lactams																			
	Amoxyclav 30 $\mu$ g/disc					Ampicillin 10 $\mu$ g/disc					Oxacillin 1 $\mu$ g/disc					Piperacillin/tazobactam 100/10 $\mu$ g/disc				
	I	II	III	IV	Mean	I	II	III	IV	Mean	I	II	III	IV	Mean	I	II	III	IV	Mean
<i>E. faecalis</i>	62	67	70	74	68.25	52	68	69	72	65.25	59	67	71	72	67.25	53	66	71	69	64.75
<i>S. aureus</i>	71	77	82	79	77.25	68	74	76	77	73.75	66	67	76	74	70.75	61	79	75	78	73.25
<i>S. pyogenes</i>	53	58	61	68	60.00	67	71	76	75	72.25	47	52	56	54	52.25	56	61	65	57	59.75
<i>A. baumannii</i>	55	62	67	65	62.25	69	77	69	59	68.50	–	–	–	–	–	47	61	64	55	56.75
<i>E. aerogenes</i>	52	61	72	66	62.75	65	69	75	71	70.00	–	–	–	–	–	65	80	81	76	75.50
<i>E. coli</i>	78	82	89	86	83.75	62	71	78	75	71.50	–	–	–	–	–	51	57	58	55	55.25
<i>K. pneumoniae</i>	54	67	76	73	67.50	74	79	83	79	78.75	–	–	–	–	–	65	72	77	73	71.75
<i>P. mirabilis</i>	25	22	31	35	28.25	32	41	43	39	38.75	–	–	–	–	–	27	31	35	35	32.00
<i>P. vulgaris</i>	42	38	41	44	41.25	34	39	48	44	41.25	–	–	–	–	–	35	42	44	45	41.50
<i>P. aeruginosa</i>	68	70	75	74	71.75	55	54	67	65	60.25	–	–	–	–	–	41	47	51	53	48.00

Note: I, April – September 2011; II, October 2011 – March 2012; III, April 2012 – September 2012; IV, November 2012 – March 2013; –, not used.

**Table 6**

Percentage of resistance of all clinical isolated bacteria to three antibiotics of cephalosporin group.

Bacterium	Cephalosporins														
	Cefepime 30 $\mu$ g/disc					Ceftazidime 30 $\mu$ g/disc					Ceftriaxone 30 $\mu$ g/disc				
	I	II	III	IV	Mean	I	II	III	IV	Mean	I	II	III	IV	Mean
<i>E. faecalis</i>	62	67	70	73	68.00	57	65	76	75	68.25	57	65	67	72	65.25
<i>S. aureus</i>	54	67	76	72	67.25	51	59	62	65	59.25	51	59	62	68	60.00
<i>S. pyogenes</i>	51	57	62	65	58.75	58	79	85	84	76.50	58	79	85	81	75.75
<i>A. baumannii</i>	45	52	57	58	53.00	52	55	61	64	58.00	52	55	59	62	57.00
<i>E. aerogenes</i>	62	70	72	69	68.25	52	57	63	67	59.75	52	57	53	56	54.50
<i>E. coli</i>	75	82	88	86	82.75	79	79	88	87	83.25	79	79	78	76	78.00
<i>K. pneumoniae</i>	64	65	71	74	68.50	54	59	63	61	59.25	54	59	63	60	59.00
<i>P. mirabilis</i>	35	32	36	39	35.50	34	43	45	49	42.75	34	43	45	51	43.25
<i>P. vulgaris</i>	45	38	44	45	43.00	36	41	43	46	41.50	36	41	43	45	41.25
<i>P. aeruginosa</i>	65	71	77	77	72.50	49	61	68	65	60.75	49	61	68	69	61.75

Note: I, April – September 2011; II, October 2011 – March 2012; III, April 2012 – September 2012; IV, November 2012 – March 2013.

**Table 7**

Percentage of resistance of all clinical isolated bacteria to four antibiotics of fluoroquinolone group.

Bacterium	Fluoroquinolones																			
	Gatifloxacin 5 $\mu$ g/disc					Levofloxacin 5 $\mu$ g/disc					Ciprofloxacin 5 $\mu$ g/disc					Ofloxacin 5 $\mu$ g/disc				
	I	II	III	IV	Mean	I	II	III	IV	Mean	I	II	III	IV	Mean	I	II	III	IV	Mean
<i>E. faecalis</i>	63	66	72	69	67.50	53	67	69	68	64.25	51	57	69	67	61.00	67	65	76	73	70.25
<i>S. aureus</i>	71	77	82	76	76.50	68	74	76	72	72.50	78	84	89	74	81.25	68	79	85	81	78.25
<i>S. pyogenes</i>	55	57	65	62	59.75	44	49	53	56	50.50	47	52	51	49	49.75	51	59	62	60	58.00
<i>A. baumannii</i>	48	54	59	59	55.00	69	77	59	54	64.75	45	52	61	77	58.75	62	67	74	71	68.50
<i>E. aerogenes</i>	47	52	51	57	51.75	65	69	75	74	70.75	72	78	81	69	75.00	52	61	63	59	58.75
<i>E. coli</i>	75	80	85	78	79.50	60	71	75	74	70.00	75	80	87	71	78.25	74	78	79	75	76.50
<i>K. pneumoniae</i>	58	62	70	68	64.50	74	79	81	80	78.50	63	71	73	79	71.50	60	68	72	72	68.00
<i>P. mirabilis</i>	25	31	29	33	29.50	37	45	46	43	42.75	35	38	45	45	40.75	31	45	47	49	43.00
<i>P. vulgaris</i>	32	38	41	45	39.00	34	39	42	41	39.00	25	34	37	39	33.75	35	37	43	41	39.00
<i>P. aeruginosa</i>	68	72	75	67	70.50	54	54	60	63	57.75	56	59	61	54	57.50	79	81	78	81	79.75

Note: I, April – September 2011; II, October 2011 – March 2012; III, April 2012 – September 2012; IV, November 2012 – March 2013.

**Table 8**

Percentage of resistance of all clinical isolated Gram-positive bacteria to two stand-alone antibiotics.

Bacterium	Stand-alone antibiotics									
	Linezolid 30 $\mu$ g/disc					Vancomycin 30 $\mu$ g/disc				
	I	II	III	IV	Mean	I	II	III	IV	Mean
<i>E. faecalis</i>	26	28	31	32	29.25	18	19	17	15	17.25
<i>S. aureus</i>	38	36	39	37	37.50	17	11	14	12	13.50
<i>S. pyogenes</i>	34	45	47	42	42.00	–	–	–	–	–

Note: I, April – September 2011; II, October 2011 – March 2012; III, April 2012 – September 2012; IV, November 2012 – March 2013; –, not used. For Gram- negatives, these two antibiotics are not used.

**Figure 1.** Colonies of *S. pyogenes* on blood agar.

#### 4. Discussion

Surgical wound-sites are generally taken care of suitably, as those wounds are planned, but burn injuries create beleaguered conditions in hospitals. Burn wounds, occurring impromptu, promote multiple infections due to the damage of the skin, the physical barrier, and cause immune suppression, independently<sup>[18]</sup>. Wounds being the invasive site for staphylococci, streptococci and enterococci among GPs, and GNs including pugnacious/PDR strains of *Acinetobacter*, *Klebsiella*, *Pseudomonas* and a few more

genera, are a priory, the prominent reckless nosocomial pathogens. Moreover, the ominous *A. baumannii* in wounds is the most rife or notorious peripatetic, etiological agent in hospital acquired infections<sup>[19]</sup>; it is a PDR pathogen<sup>[5,14,20]</sup>, affording much difficulty for its control.

For the control of wounds, topical antibacterial agents are used in addition to systemic ones. The popular topical antimicrobial, Acticoat with the silver sulfadiazine influences the respiratory chain at the cytochrome level, by disrupting the microbial electron transport system mediated by the silver ion<sup>[21]</sup>. In fact, because of the slow release of silver ions from Acticoat, a stronger and longer antimicrobial effect had been recorded, compared to the other classical silver containing topical agents. Thus, it was found as the more effective agent against aerobic, anaerobic GPs and GNs, fungi, and viruses on wound sites<sup>[21]</sup>. Another broad spectrum topical antibiotic obtained from *Pseudomonas fluorescens*, mupirocin had been recorded strongly inhibiting protein and RNA synthesis in pathogens<sup>[22]</sup>. Moreover, *A. baumannii* in burn injury infections causes delay in the wound healing process, probably due to its ability to cause systemic circulation; sepsis mostly occurs ending with high mortality rates with patients who were inadequately treated<sup>[20,23]</sup>. Acticoat, however, was independently reported to control MDR *A. baumannii*, *in vivo* by other workers<sup>[18,24]</sup>. Octanidiene dihydrochloride is another topical antibacterial agent used in the control of active and chronic wounds<sup>[23,25]</sup>

without any disadvantage of the other chemical, silver sulfadiazine, as the later has a lower escher–penetration rate, needing frequent applications, whereas octenidine dihydrochloride based formulations are effective against GPs, GNs and fungi[26]. Quintessentially, Acticoat had appreciable control actions over octanidiene dihydrochloride or chlorhexidine acetate on *A. baumannii*[23]. Sofra–tulle (framycetin sulphate bp 1%), framzyne are sulfamylone creams, also are used as topical applications for burn injuries with *Pseudomonas* infections. Indeed, aggressive uses of antibiotics lead to conditions for the growth of fungi in burns; *Candida albicans* is the most common associated fungal pathogen[27]. Powdered sulfonamides, as well as, its cream–based formulations had been a popular topical medicine for burn wounds, with a limited absorption of sulfonamides at injured sites. Further, in children, agranulocytes are induced with another control agent, sulfapyridine, when applied to burn injuries. Therefore, sulfonamide creams are rarely used for burn injuries. Nevertheless, sulfadiazine are less readily absorbed in the body in comparison to sulfonamides[28].

GP bacteria particularly survive the thermals of burning, unless an immediate topical treatment is done around 48 hours; staphylococci come up from the sweat glands and hair follicles colonizing at the burn site[29]. In a typical study, from surgical wounds analyzed with 343 patients, *S. aureus* was prevalent at 28.2%, *P. aeruginosa* at 25.32%, *E. coli* at 7.8%, *S. epidermidis* at 7.1% and *E. faecalis* at 5.6%[30]. *Proteus* sp. particularly was reported to have been isolated from an Indian tertiary care hospital; all the isolated strains of pathogens were resistant to the entire antibiotics group used, among which, *S. aureus* in GPs, *A. baumannii* and *P. aeruginosa* among GNs had the maximum resistance values[31]. In a study from Netherlands, it was reported that a cohort of 11 patients and nurses of the surgical ward of a hospital were infected with *S. pyogenes* with a particular serotype of low virulence; another group of patients from the same hospital had infection with another serotype of *S. pyogenes* causing heavy delay in wound healing[32]. This study clearly demonstrated the outbreak of *S. pyogenes* infections in hospital settings.

Apart from surgical wounds, post–operative peritonitis is an important determinant in toxic shock syndrome in ICU patients. In a study from Paris, it was found that GP bacteria were prevalent in 40% of cases and *E. faecium* was the leading organism (19%), followed by streptococci (11%), *S. aureus* (3%), coagulase negative staphylococci (8%). Similarly, among infections from GNs, Enterobacteriaceae members contribute 37% of the total infection; *E. coli* were 18%, and the rest other bacteria were as follows: *Enterobacter* 8%, *Klebsiella* 5%, *Morganella* 3%, *Proteus* 2%, *Citrobacter* 2%;

*Pseudomonas* 6% and *A. baumannii* 1%. The prevalence of the total anaerobic bacteria causing post–operative peritonitis in this study was 13% with the predominance of *Bacteroides* sp. Among all these bacteria, the prevalence of MDR Enterobacteriaceae was 5% and that of *P. aeruginosa* was 2%[33]. An Indian study, however, recorded increasing incidences of MDR *Klebsiella* sp., isolated from 72% infected hospitalized patients, out of which, 3.44% isolates were capable of the production of *Klebsiella pneumoniae* carbapenemase enzyme, of which 26 strains from hospital and 8 strains were from community isolates[34]. From Greece, MDR GN bacteria were reported from ICU surgical patients; it was reported that hospitalization for more than 5 days increased the susceptibility towards MDR infections[13]. Sternal wound infections after cardiac surgery were caused by MRSA and GNs in 22.4%, within two years of study with 1895 patients in France[35]. It is consensus that a patient admitted to a hospital, more often with resource–limited settings, for a specific problem or even due to problems of old age, picks up one or more MDR infectious bacteria. Risk–factors of post–discharge invasive infection from MRSA in hospitalized patients can be imagined, as monitored with MRSA in the USA through BSI for its high rates of antibiotic resistance, for example[36].

It is believed that antibiotics are symbol and substance of clinical management today, and it is hard to think of an aspect of contemporary life that does not depend on antibiotics. Taking to surprise, nosocomial spread of MDR bacteria has become so commonplace that clinicians of both developing and developed countries get often emotionally grieved during treatment to delirious hospitalized patients of any ailment, with whom an infection on hospitalization with an MDR bacterium could be a terminal illness. As known, several factors contribute to the problem of the emergence of MDR bacteria: (1) Device–and–fomite–associated nosocomial infections are frequent in hospital settings everywhere and always[37], with begrimed settings. (2) The drug resistance character has ramifications: bacteria may be resistance to representative, frequently used antibiotics of several classes; paradigmatically, the production of extended spectrum  $\beta$ –lactamase has rendered resistance to antibiotics of penicillin/cephalosporin group[38]; carbapenemase production affords resistance in GN bacteria to carbapenems (imipenem, meropenem and ertapenem) in use. (3) Mutation rates in bacteria are faster because of simple, plastic genomes — one mutant cell in 106 to 108 bacterial cells in the presence of an antibiotic–stress is known to be drug resistant[39]. (4) To avoid host toxicity, antibiotics doses are mostly fixed at some lower concentration that is often below the mutant preventive concentration [40], giving ways to the development of mutants. (5) Genetic recombination

mechanisms (bacterial transformation and conjugation) are operative in natural systems, such as hospital sewages, facilitating the creation of a pool of drug resistant characters in camaraderie MDR strains<sup>[41]</sup>. (6) Absence of a stringent antibiotic policy triggers the emergence of resistant bacteria as both clinicians and patients use antibiotics belonging to higher generations without often being warranted. (7) Patients, particularly, often do not complete the course of prescribed dose of an antibiotic, because of the blithesome effect of the control of infection from the start of the course. Thus, bacterial resistance to antibiotics is a consistently complex and dynamic affair, involving major genetic and biochemical mechanisms, bacterial transformations, interchange of integrons, hypermutability, plasmids mediated improvements in resistance factors, ending with drug efflux mechanism and gaining of characters to synthesize indigenous and exogenous antibiotic degrading enzymes.

This study demonstrated that most of these pathogens isolated from clinical samples were MDR and they are potential enough to destroy the clinical totem pole of a hospital and to precipitate devastating episodes in the community. As analyzed, suppurative infections are one of the major problems of health, as MDR bacteria could attack several organs such as lungs, heart and kidneys, through BSI. Compared to infection studies on suppurative pathogens from other hospitals globally, the surveillance data of this typical hospital clearly gives high values of wound infections among which, the MRSA infection predominates. Burn injuries are needed to be attended more stringently in this peninsula too. Steps to prevent invasive infections of most of these pathogens, particularly MRSA in communities must be started in the hospital with proper/ prior post-discharge settings.

### Conflicts of interests

The authors declare that they have no conflict of interests.

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