Material and Methods

The setting

Guwahati Medical College Hospital is a typical government run teaching hospital in the north-eastern state of Assam. The hospital is a 5 storied concrete building accommodating 12 speciality and 11 super-speciality departments (Medicine, Gastroenterology, Haematology, Endocrinology, Tb & chest disease, Psychiatry, Paediatric, Neurology, Cardiology, Surgery, Orthopaedics, Neurosurgery, Paediatric Surgery, Plastic Surgery, Nephrology, Urology, Cardiothoracic Surgery, Obstetrics & Gynaecology, ENT, Intensive Care Unit, Intensive Therapy Unit, Paediatric Intensive Care Unit, Neurosurgery Intensive Care Unit and Radiology) with a total bed capacity of 1500.

The setting is different from that of the hospitals in developed countries in two important aspects viz., there is no formal control of hospital infection programme and antimicrobial prescribing is mostly empirical.

Bacterial isolates

Isolates of MRSA (n=27), MSSA (n=18), ESBL producing species of Enterobacteriaceae (n=65) and non-ESBL producing species of Enterobacteriaceae (n=19) were collected from 80 surgical (three units), 29 orthopaedics (one unit) and 73 medical (four units) male patients on a single day between 21st July and 1st August 2001.

Dry swabs were used to collect specimens from two carriage sites viz., nose and throat and any wound sites accessible without removing the bandage and dressings.

Isolates were categorised as nosocomial or community, respectively based on whether from patients with ≥48 or ≤48h admission to the hospital.
Case-control study

A case control study was performed to determine the individual risk factors of acquiring resistant pathogens whilst in the hospital.

Baseline demographic and clinical data such as diagnoses, duration of inpatient stay, procedures performed and antimicrobials prescribed with duration were recorded from the patient’s medical record by the author.

The cases were defined as subjects colonised infected with either with (1) MRSA or (2) ESBL producing species of Enterobacteriaceae and (3) both. The controls were not colonised with any of these pathogens and selected randomly to avoid any selection bias using SPSS (version 10) from the cohort in equal numbers to the cases in each category.

The risk factors for acquisition of resistant bacteria studied are exposure to three antimicrobial classes viz., (1) third generation cephalosporins, (2) aminoglycosides and (3) fluoroquinolones, (4) any surgical intervention during the admission, and (5) the duration of inpatient hospital stay (≥48h or ≤48h). Most multi-resistant bacterial isolates were hospital acquired i.e., isolated from patients with ≥48h of hospital stay, therefore, duration of inpatient stay was included as a risk factor in the study. Age was not considered as a risk factor as the cohort was relatively uniform and young in age.

Laboratory methods

Isolation of S. aureus: The swabs were plated on to mannitol salt agar and incubated overnight. The suspect yellow colonies of S. aureus on the mannitol agar plate that agglutinated with Pastorex (Sanofi Diagnostics Pasteur Ltd., France) were picked, obtained in pure culture after overnight incubation on nutrient agar and processed.

Isolation of Species of Enterobacteriaceae: The swabs were plated on to MacConkey agar and incubated overnight. Typical lactose-fermenting and non-fermenting colonies of
Enterobacteriaceae were picked, obtained in pure culture after overnight incubation on nutrient agar and processed.

A collection of CoNS, Pseudomonas, Acinetobacter and Enterococcal isolates were sloped for further work, although, the antibiogram of CoNS isolates are presented in this study.

**Standardised disc sensitivity method (British Society of Antimicrobial Chemotherapy)**

*Preparation of plates:* Iso-Sensitest agar (ISA, CM471, Oxoid, UK) was prepared according to the manufacturer's instructions. For the detection of meticillin resistance in Staphylococci Columbia agar (Oxoid CM331) with 2% NaCl was used. 25 ml molten agar was poured into 90 mm sterile Petri-dishes to give a mean depth of 4.0 mm ± 0.5 mm. Plates were dried to remove excess moisture from the surface of the agar plates and stored in vented plastic boxes at 8-10°C prior to use.

*Preparation of inocula:* Colonies were taken directly from the plate into sterile distilled water to make a suspension ≥ 0.5 McFarland standard. The suspension was then adjusted to the 0.5 McFarland standards, if necessary, by adding sterile distilled water to be used within 10 minutes. Before inoculation, this suspension equivalent to a 0.5 McFarland standard was diluted in distilled water to: 100 (Enterobacteriaceae, *Pseudomonas* sp, *Acinetobacter* sp) or 1:10 (Staphylococci, Enterococci, Serratia sp) as appropriate to be used within 15 min of preparation.

*Inoculation of agar plates:* The adjusted suspension was inoculated within 15 min onto plates by dipping a sterile cotton wool swab into the suspension and removing the excess by turning the swab against the side of the container and spreading evenly over the entire surface of the plate by means of a rotary platter. The plate was allowed to dry before applying discs within 15 min of inoculation.
**Application of discs:** Discs (Oxoid®, Oxoid Ltd, England) are firmly applied to the surface of an agar plate by using a disc dispenser (6 discs on 90-mm plate). All discs were stored < 8°C to prevent loss of potency prior to use in sealed containers with a desiccant and protected from light.

**Incubation:** Plates were incubated at 35-37°C in air for 18-20h (24h for enterococci).

**Control:** *E. coli* NCTC 12241 (ATCC 25922) or NCTC 10418; *E. coli* NCTC 11560; *S. aureus* NCTC 12981 (ATCC 25923) or NCTC 6571; *S. aureus* NCTC 12493; *P. aeruginosa* NCTC 12934 (ATCC 27853) or NCTC 10662; *E. faecalis* NCTC 12697 (ATCC 29212).

**Measurement of zone of inhibition:** Diameter of zones of inhibition was measured (mm) with the edge taken as the point of inhibition as judged by the naked eye of the control strain and test with a ruler.

**Identification and detection of meticillin resistance**

All *S. aureus* isolates are confirmed by *tube coagulase test*.

**Medium:** Columbia (Oxoid®, Oxoid Ltd, England) or Mueller-Hinton agar (Oxoid®, Oxoid Ltd, England) following the manufacturer's instructions with 2% NaCl added to it. After autoclaving, mixed well to distribute the sodium chloride and poured on to the plates to give a depth of 4 mm in a 90 mm sterile Petri-dish (25 ml).

**Inoculum:** Inoculum was prepared as above.

**Control:** Susceptible controls (ATCC 25923 or NCTC 6571) to test disc content. NCTC 12493 is a resistant strain used to check that the test will detect resistant strains.

**Discs:** Oxacillin 1µg disc.

**Incubation:** Incubated for 24 h at 30°C.
**Reading and Interpretation**: Measured as detailed above. Colonies growing within zones were identified and re-tested for resistance to Meticillin/ oxacillin. Interpretation is as follows: susceptible $\geq 15$ mm diameter, resistant $\leq 14$ mm diameter.

**Antibiogram-Resistogram (AR) typing**

Isolates were tested against a range of antibiotics with a view to generate a profile for interpretive reading for recognising the unusual and interfering resistance mechanism.$^{304}$

**Typing of MRSA**

MRSA isolates were investigated by antibiogram-resistogram, phage typing and $SmaI$ DNA macrorestriction analysis (PFGE).

**Phage typing (S. aureus)**

Phage typing was carried out by the Laboratory of Hospital Infections, Central Public laboratory Services (CPHLS), London NW9 5HT. Isolates were typed using 23 (Group I: 29, 52, 52A, 79, 80; Group II: 3A, 3C, 55, 71; Group III: 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85; Group V: 94, 96; Group M: 81, 95) of Basic International Set at 100 x the RTD plus the four experimental phages from the UK, phages 88A, 90, 83C and 932.$^{305}$ Reactions were read as inhibition ($=0$), weak reaction ($=2$, $3$) and strong reactions ($=8$, 9). Existing two-reaction difference rule was applied to both Meticillin sensitive and resistant isolates of $S. aureus$.$^{306}$

**PFGE typing**

A few representative MRSA isolates of indistinguishable phage pattern were typed by PFGE by the CPHLS, London.

**Identification of Enterobacteriaceae**

The LOGIC identification system$^{307, 308}$ (six conventional biochemical tests to produce an identification scheme, see Table 17 and 18) was used to identify Enterobacteriaceae.
Preparation of the media

*Decarboxylase media* were prepared from dehydrated Moeller decarboxylase base (Difco) according to the manufacturer’s instructions. Following the addition of amino acid the pH was adjusted to 6.2.

*Peptone water sugars* were prepared containing 1% sugar- either glucose (BDH) or cellibiose (BDH), 1% proteose peptone (Oxoid), 0.5% sodium chloride. Bromothymol blue was used as an indicator and the pH was adjusted to 7.6.

*Tryptone water* was prepared from dehydrated tryptone base (LAB M) according to manufacturer’s instructions.

*Urea broth* was prepared by Christensen’s method, which uses 2% urea (Oxoid).

<table>
<thead>
<tr>
<th>Table 17 Interpretation of LOGIC test results</th>
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<tr>
<td><strong>Test</strong></td>
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<tr>
<td>Lysine</td>
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<td>Ornithine</td>
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<td>Glucose</td>
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<td>Indole</td>
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<td>Celiobiose</td>
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<td>Urea</td>
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Six plastic tubes were assembled each containing eight drops of substrate (tube 1- lysine, tube 2-ornithine, tube 3-glucose, tube 4-tryptone water, tube 5-cellobiose and tube 6-urea solution). An equivalent of 4-5 medium sized colonies was then removed from DST agar and emulsified in 0.4 ml physiological saline to produce a thick suspension. One drop of this suspension was then added to each of the 6 tubes and sterile mineral oil added to tubes containing lysine, ornithine, glucose and urea. All tubes were incubated at 37°C in the hot room.

After 4 hours of incubation 4 drops of Kovac’s reagent were added to the tube containing tryptone water to detect indol production. Any of the remaining tubes showing weak or doubtful colour changes was reincubated for another hour and then interpreted.
To reduce the possibility of misidentification, bacteria identified as *Citrobacter freundii* must also be confirmed as lactose fermenting on the original CLED/MacConkey isolation plate. Similarly, *Providentia* or *Serratia* sp must be confirmed as lactose negative. This reduces the risk of misidentifying indole and decarboxylase negative *E. coli*, respectively. *Serratia* sp were confirmed as being negative to poly-salmonella ‘O’ antigen to distinguish them from *Salmonella* sp.

**Table 18 List of acceptable LOGIC profile**

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<thead>
<tr>
<th>Biochemical profile</th>
<th>Identification</th>
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<td>Lysine Ornithine Glucose Indole Cellobiose Urea</td>
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A few isolates that did not give acceptable LOGIC profile were identified by API 20E (Biomérieux, Marcy l’Etoile, France).

**Detection of ESBL in *E. coli* and Klebsiella**

(I) **Double-Disc Synergy Test**- DDST: A plate was inoculated with the test isolate, as for routine British Society of Antimicrobial Chemotherapy (BSAC) disc susceptibility test. Discs containing ceftazidime 30 μg and cefotaxime 30 μg were applied on either side (25-30 mm apart) of a coamoxyclov 20 + 10 μg disc. The plate was incubated overnight at 37 C, and ESBL production was inferred when the cephalosporin zone is expanded by the clavulanate.

(II) **Etest ESBL strips**: Etest (AB Biodisk, Solna, Sweden; Cambridge Diagnostic Services, Cambridge, UK) with a ceftazidime gradient at one end and a ceftazidime plus clavulanate
gradient at the other were used to detect ESBLs according to manufacturer's instructions (0.5 MacFarland). If the ratio of the MIC of ceftazidime to the ratio of ceftazidime with clavulanate was $\geq 8$, ESBL production was inferred.

(III) **Combined disc method:** A plate was inoculated with the test isolate, as for routine British Society of Antimicrobial Chemotherapy (BSAC) disc susceptibility with discs containing an extended spectrum cephalosporin *viz.* cefpodoxime 10 $\mu$g and cefpodoxime + clavulanate 10 + 1 $\mu$g. The plate was incubated overnight at $37^\circ$ C, and ESBL production was inferred when the zone given by the disc containing cefpodoxime + clavulanate 10 + 1 $\mu$g are $\geq 5$ mm larger than those without the inhibitor *viz.*, clavulanate (See figure 11).
Double-disc diffusion: *K. pneumoniae* TEM-10-

**Cefotaxime 30 μg**
**Cefotaxime + clavulanate**

**Augmentin 20-10**

**E-test**

Detection of Inducible AmpC

**Cefotaxime**
**Cefotaxime + clavulanate**

**Figure 11 Detection of ESBL and AmpC**
Detection of inducible chromosomal Amp C β-lactamase

A plate was inoculated with the test isolate, as for routine British Society of Antimicrobial Chemotherapy (BSAC) disc susceptibility test, and cefotaxime 30 μg and cefoxitin 30 μg discs were applied 25 mm apart. Inducible chromosomal β-lactamase production was inferred by the blunting of the cefotaxime zone adjacent to the cefoxitin disc. (See figure 11)

Detection of ESBL in enterobacter species

Modified Double-Disk Synergy Test- DDST: A plate was inoculated with the test isolate, as for routine British Society of Antimicrobial Chemotherapy (BSAC) disc susceptibility test, and discs containing cefepime 30 μg and co-amoxyclyv30 + 10 μg were applied 20 mm apart. The greater efficacy of cefepime (and cefpirome) instead of extended spectrum cephalosporin (e.g., ceftazidime or cefotaxime) in double disc ESBL detection test is expected, since the former, but not the latter, retain activity against derepressed variants of enterobacters. Proximal placement of disc (20 mm) from the coamoxyclyv disc is more successful than discs placed at the standard distance of 30 mm.

Calculation of antimicrobial consumption

A defined daily dose is the assumed average maintenance dose per day for a drug used for its main indication in adults (World Health Organisation). The number of DDDs for each antibiotic is determined by a group of experts from the WHO and is a technical unit of measurement and not necessarily a measure of good practice.

Actual antibiotic consumption data on the day of survey were collected from patient’s medical notes and expressed as a number of Defined Daily Doses (DDD) per 100 bed-days using ‘ABC Calc’ as defined by the WHO Collaborating Centre for Drug Statistics Methodology, Oslo (Norway).
Unit-dose: Depending on the product, a unit dose corresponds to one tablet, one gelcap, one vial of infusion solution, one vial of mixture, etc. Package: The package was equal to the unit dose as drugs were distributed individually (number of unit doses per package is 1).

Number of bed-days (during a specific period): Calculated directly (= number of patients on the day of survey). A bed-day corresponds to one occupied hospital bed during one day.

Observations of Infection Control practices

The inspection of the hospital was carried in August 2008 out to assess the facilities and current state of affairs in reference to hand hygiene, safe clinical practices, blood and injection safety, decontamination and sterilization, provision of safe water, sanitation and health care waste management. The medical and surgical wards, operation theatre, sterile services facility were visited and the build environment was assessed in terms of the infrastructure and facilities. Infection control practices carried out by the staff were observed and staff awareness was assessed through informal interactions. The hospital organisational structure was considered to see if there was role and responsibilities for infection control were allocated.

Data from a teaching DGH in England

Data were collected from a teaching District General Hospital (DGH) in the northeast of England to compare and contrast and inform discussion of the results of the present study. The author was involved in generating these data for clinical audit purposes in this hospital as a member of the staff.