Virulence 4:2, 172–184; February 15, 2013; © 2013 Landes Bioscience

# Early diagnosis of resistant pathogens
## How can it improve antimicrobial treatment?

Sanjay Bhattacharya

Consultant Microbiologist; Tata Medical Center; Kolkata, India

**Keywords:** MDROs (multidrug-resistant organisms), early diagnosis, surveillance culture, PCR, infection control, antimicrobial therapy, MRSA, ESBL, carbapenem resistance, VRE

Infections with organisms that are resistant to various antimicrobial agents pose a serious challenge to effective management of infections. Resistance to antimicrobial agents, which may be intrinsic or acquired, has been noted in a wide variety of microorganisms causing human infections. These include resistance to antiviral agents in HIV, HBV, CMV and influenza virus, anti-parasitic agents in *Plasmodium falciparum*, anti-fungal agents in certain *Candida* species and MDR (multidrug-resistant) tuberculosis. It is however, the problem of multidrug-resistant bacterial infections (caused by MRSA, VRE, ESBL/AmpC/metallo-β-lactamase producers and colistin-resistant Gram-negative bacilli) that has become a cause of major concern in clinical settings. Infections with these organisms can increase morbidity, mortality, increase the cost of therapy and increase the duration of hospitalization. The objective of this article is to review the question how early diagnosis of these infections, affects the overall management of infected or colonized patients, with regard to antimicrobial therapy.

### Introduction

Infection due to multidrug-resistant organisms (MDROs) is a major concern in clinical management. Multidrug-resistant bacteria can infect or colonize young or old, sick or healthy, immuno-competent and immuno-compromised individuals. Colonization as the term implies, may not manifest as a clinically overt infection. However, both in immuno-competent as well as in immuno-compromised individuals, with or without other associated co-morbidities, MDROs may manifest as life threatening infections. The purpose of this review is to discuss how early detection of resistant pathogens improves overall anti-infective management including anti-microbial therapy.

Antimicrobial resistance, either intrinsic or acquired, has a significant bearing on the pathway of clinical management of infected or colonized patients. This may manifest as a clinical resistance to treatment with antimicrobial agents, a microbiologically demonstrable phenotypic resistance (with an increase in the minimum concentration of antimicrobial agent required to inhibit the growth of a microbe), or a genotypic resistance (where specific antimicrobial resistance genes are demonstrable by molecular techniques such as PCR, hybridization and DNA sequencing). In some cases these three types of resistances may coexist, but not necessarily in all. The causes and mechanisms of antimicrobial resistances are multi-factorial, and they have been well documented in a wide variety of microorganisms ranging from viruses [e.g., human immuno-deficiency virus (HIV), hepatitis B virus (HBV), cytomegalovirus (CMV) and influenza virus], parasites (e.g., *Plasmodium falciparum*), fungi (e.g., azole resistance in *Candida glabrata* and *Candida krusei*, amphotericin resistance in *Aspergillus terreus*, caspofungin resistance in *Candida parapsilosis*, an emerging multi-drug resistance in *Candida haemulonii* and multi-drug resistance in tuberculosis). However, it is in the field of bacterial infections that the problem of multidrug-resistant organisms (MDROs) has been most acutely faced especially in acute care and critical care settings.

Correspondence to: Sanjay Bhattacharya; Email: drsanjay1970@hotmail.com
Submitted: 08/06/12; Revised: 12/03/12; Accepted: 12/18/12
http://dx.doi.org/10.4161/viru.23326

### Epidemiology of Multidrug-Resistant Organisms (MDROs)

The number of infections due to MDROs is increasing. Outbreaks of infection have been reported with MDROs as for example the multidrug-resistant *Serratia marcescens* outbreak in a Neonatal Intensive Care Unit at Baltimore where 18 neonates were infected. Transient carriage on the hands of healthcare personnel or on respiratory care equipment could have been the likely mode of transmission, and environmental reservoirs (such as wash sink) may act as potential reservoirs of infection. Cohorting patients and staff, bed closures and introduction of additional personnel interrupted transmission and halted the outbreak.[1]

The reasons for the increase in incidence and prevalence of MDROs are multi-factorial. Regions of the world which have a high prevalence of MDROs among patients and general population groups are more likely to see an increase in the incidence of such infections. MDROs could be present as a reservoir of infection in different milieu which include: endogenous colonization, exogenous spread from hospital environment having a high burden of MDRO patients, ground water or other water sources contaminated because of inadequate waste management or sewage disposal system, acquisition of MDRO in the community because of indiscriminate use of antibiotics available over the

counter (especially in developing countries) and contamination of food and rarely medicines.

There is evidence to show a correlation between antibiotic exposure and subsequent development of antibiotic-resistant bacteria. Conversely it has also been shown in a study from Peru that residence in a zone where a larger proportion of households consumed home-raised chicken (as opposed to intensively antibiotic-raised market chicken) protected against carrying *E. coli* resistant to all drugs.[2]

Globally the reported prevalence and incidence of MDRO is variable, and depends on the MDRO type, method of detection, sampling and testing strategy, and publication/ reporting bias. Under-reporting of MDRO is a possibility especially if publication of MDRO status of a hospital is seen as a threat to business (a phenomenon which may be observed in private health care industries) or reputation of the hospital. Over-reporting is also possible if robust methodologies are not followed, and repeat isolations from same patient is not de-duplicated. The prevalence of MDROs would be high in any country where the factors contributing to its transmission and reservoir development are not controlled. Many MDRO genes such as the ones responsible for ESBL (extended spectrum β-lactamase) production are plasmid mediated, and can spread between bacterial species contributing to increased incidence and prevalence.[3]

## Clinical Impact of MDROs

The association of multidrug-resistant organisms (MDROs) and clinical outcome was well documented in a retrospective cohort study in New York, which looked at specific factors associated with positive outcomes in bacteremia due to carbapenem-resistant *Klebsiella pneumoniae* (CRKP). The failure of microbiologic eradication at 7 d (i.e., persistence of positive blood culture despite appropriate therapy) was independently associated with 30-d mortality. Additional measures such as source control and microbiologic eradication at 7 d were associated with a favorable prognosis at 7 d. Notably breakthrough bacteremia occurred in those patients receiving tigecycline therapy (a bacteriostatic agent).[4]

It was reported in a study from Israel that the crude and attributable mortality rates associated with carbapenem-resistant *K. pneumoniae* bacteremia (CRKPB) were significantly more than control subjects without bacteremia. It was noted that patients with CRKPB were significantly more likely than those without bacteremia to require care in an intensive care unit support (37.5% vs. 9.4%), ventilator support (53.1% vs. 25%), and use of a central venous catheter (59.4% vs. 28.1%). The crude mortality rate was significantly higher for those with CRKPB than for control patients (71.9% vs. 21.9%). Attributable mortality due to CRKPB was high 50% [95% confidence interval (CI), 15.3–98.6%]. A mortality risk ratio of 3.3 (95% CI, 2.9–28.5) was found for case subjects with carbapenem-resistant *K. pneumoniae* bacteremia. Not surprisingly, patients with CRKPB required more intensive care and invasive care.[5]

In some cases carbapenem-resistant *K. pneumoniae* infection (CRKPI) has been recognized in patients receiving highly intensive interventions. In a study from New York CRKPI was independently associated with recent organ or stem-cell transplantation, receipt of mechanical ventilation, longer length of hospital stay, and exposure to antibiotics such as cephalosporins and carbapenems. Significantly, case patients (i.e., those with CRKPI) were more likely than control patients to die during hospitalization (48% vs. 20%) and to die from infection (38% vs. 12%). Focus of infection removal (i.e., by drainage or debridement) was independently associated with patient survival.[6]

There is uncertainty whether the multidrug-resistant organisms (MDROs) are more virulent than their drug sensitive counterparts. The general view has been that the drug-resistant strains are not necessarily more virulent per se, and that the difference in clinical outcome may be attributable to the restricted therapeutic options available for their treatment, failure of empirical treatment, and the delay in instituting effective therapy. However, the findings of a study suggested that at least in certain cases, the organism itself may be contributing to the poor prognosis. A prospective observational study of adult inpatients in Michigan with *Staphylococcus aureus* bacteremia attempted to shed some light on this question by performing SCCmec typing of methicillin-resistant *S. aureus* (MRSA) isolates. In this cohort, the illness severity index was similar in MRSA and methicillin-sensitive *S. aureus* (MSSA) cases. MRSA was found to cause greater in-hospital mortality (23.9% vs. 8.9%), longer duration of bacteremia (4.7 d vs. 2.7 d), but similar rates of metastatic infection (14.7% vs. 15.6%). By multi-variate analysis it was observed that SCCmec type II caused highest mortality (33.3%) vs. type IVa (13.5%), other MRSA types (12.5%) and MSSA (8.9%). SCCmec IVa resulted in the highest metastatic infection [26.9% vs. 9.1% (SCCmec II), 8.3% (other MRSA) and 15.6% (MSSA)]. SCCmec II was found to be a predictor of mortality [odds ratio (OR) = 3.73; 95% confidence interval (CI) = 1.81 to 7.66], SCCmec IVa was seen as a predictor of metastatic infection (OR = 3.52; CI = 1.50 to 8.23) and MRSA (independent of SCCmec type) was a predictor of persistent bacteremia (OR = 4.16; CI = 1.47 to 11.73).[7] The application of whole genome sequencing and gene expression profile in resistant vs. sensitive strains might help in providing a greater insight into pathogenesis of infections caused by MDROs.

Inappropriate initial antimicrobial therapy (IIAT) has been associated with decreased survival in patients with health care-associated pneumonia (HCAP). Use of antibiotics covering all the potential pathogens would improve prognosis. A study from Missouri demonstrated that empiric anti-pseudomonal antibiotics and empiric anti-MRSA antibiotics were independent predictors of appropriate therapy for HCAP.[8] Another retrospective study of 18,209 patients from Seattle showed a correlation with the timing of antibiotic administration and outcomes for patients hospitalized with community-acquired pneumonia. Antibiotic administration within 4 h of arrival at the hospital was associated with reduced in-hospital mortality, reduced mortality within 30 d of admission and reduced length of stay (LOS) exceeding the 5-d median. Mean LOS was 0.4 d shorter with antibiotic administered within 4 h than with later administration.[9] A single center cohort study from Pennsylvania showed

**Table 1.** Antibiotic options for the treatment of MDROs (multidrug-resistant organisms)

| MDRO type | Resistance pattern | Therapeutic options |
|---|---|---|
| MRSA | R to all β-lactam antibiotics—penicillins, cephalosporins, carbapenems | Glycopeptides (e.g., vancomycin or teicoplanin), oxazolidinone (e.g., linezolid), glycylcycline (e.g., tigecycline) and lipopeptide (e.g., daptomycin) |
| VRE | R to glycopeptides | Oxazolidinone (e.g., linezolid), glycylcycline (e.g., tigecycline) and lipopeptide (e.g., daptomycin) |
| ESBL | R to all cephalosporins and aztreonam | Carbapenems (e.g., imipenem, meropenem, ertapenem), aminoglycosides (e.g., gentamicin, amikacin based on susceptibility), BL-BLI (e.g., piperacillin-tazobactam in selected cases), polymyxin (e.g., colistin) and glycylcycline (e.g., tigecycline) |
| AmpC | Inducible cephalosporin resistance | Carbapenems (e.g., imipenem, meropenem), polymyxin (e.g., colistin) and glycylcycline (e.g., tigecycline) |
| MBL | R to carbapenems, penicillins, cephalosporins | Polymyxin (e.g., colistin) and glycylcycline (e.g., tigecycline) |
| Colistin R GNB | R to carbapenems, penicillins, cephalosporins, polymyxins | Glycylcycline (e.g., tigecycline, in few cases), fosfomycin (in few cases), no effective agent for serious systemic infections in neutropenic patients |

R, resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant Enterococci; ESBL, extended spectrum β-lactamase producers; MBL, metallo-β-lactamase or carbapenemase producers; GNB, Gram-negative bacilli; BL-BLI, β-lactam + β-lactamase inhibitor.

that elapsed times from triage and qualification for early goal-directed therapy to administration of appropriate antimicrobials were primary determinants of mortality in patients with severe sepsis and septic shock. A significant association was seen at the ≤ 1 h time cutoff (mortality 25.0 vs. 38.5%).[10] However, the desire for early administration of appropriate antibiotic therapy may sometimes paradoxically lead to misdiagnosis and inappropriate utilization of antibiotics. In a study from the US done after the publication of the 2003 Infectious Diseases Society of America guidelines for community-acquired pneumonia (CAP) (which recommended the initiation of antibiotic therapy within 4 h of hospitalization), it was observed that more patients had a hospital admission diagnosis of CAP without radiographic abnormalities (28.5% vs. 20.6%), although more patients received antibiotics within 4 h of triage (65.8% vs. 53.8%). The final diagnosis of CAP dropped to 58.9% in 2005 from 75.9% in 2003. There were no significant differences in pneumonia severity index or CURB-65 (confusion, urea, respiratory rate, BP and age  65 y) scores, or mortality.[11]

## Rationale of Doing Surveillance Microbiology

**The impact of multi-faceted infection control interventions.** The gut is the reservoir of many bacteria which in the intestine live as harmless commensals in healthy individuals. Gram-negative bacilli of the Enterobacteriaceae family (e.g., *Escherichia coli*, *Klebsiella* species, *Enterobacter* species, *Serratia* species, *Citrobacter* species, *Proteus* species, *Providencia* species and *Morganella* species) and *Enterococcus* species (*Enterococcus faecalis* and *E. faecium*) and anaerobes colonize the intestine in large numbers. The first two groups (coliforms and enterococci) bacteria are also the common organisms where antibiotic resistance may develop. The common types of antibiotic resistance bacteria that could be detected in the gut microbes include extended spectrum β-lactamase producers, carbapenem-resistant Enterobacteriaceae, vancomycin-resistant Enterococci, ampicillin and high level aminoglycoside resistant Enterococci. The understanding is that the identification of resistant organisms by surveillance microbiology would aid in more informed selection of anti-microbial agents for empirical therapy, and institution of timely infection control measures.

Active surveillance is useful if it is part of a multi-factorial intervention to control MDROs. In a study from Tel Hashomer, Israel, it was observed that 56% of patients whose clinical microbiologic culture results were positive for carbapenem-resistant *K. pneumoniae* developed a nosocomial infection. During the observation and intervention period, the rate of carbapenem-resistant *K. pneumoniae* rectal colonization was 9%. Fifty-two percent of carbapenem-resistant *K. pneumoniae*, were identified by screening cultures. Thirty-eight percent of days of contact precautions were added as a result of active surveillance. After initiation of infection control measures, there was observed a significant decrease in the incidence of carbapenem-resistant *K. pneumoniae* infection.[12]

The effect of enhanced infection control measures with screening for gastrointestinal colonization on limiting the spread of carbapenem-resistant *Klebsiella pneumoniae* in a New York City hospital was assessed in a retrospective observational study. In it in addition to being placed in contact isolation, all patients colonized or infected with carbapenem-resistant Gram-negative bacilli, vancomycin-resistant Enterococcus or methicillin-resistant *Staphylococcus aureus* were cohorted to one end of the unit as part of infection control precautions. Improved decontamination of hands and environmental surfaces was also encouraged and performed. Routine rectal surveillance cultures were screened for the presence of carbapenem-resistant pathogens. It was observed that the mean number of new patients per 1,000 patient-days per quarter with cultures yielding carbapenem-resistant *K. pneumoniae* decreased from 9.7 before the intervention to 3.7 after the intervention.[13]

**Role of surveillance culture to guide empirical antibiotic therapy.** Timely initiation of appropriate antibiotic therapy is crucial for management of severe infection (**Table 1**). Appropriate antibiotic therapy could get delayed for infections caused by

antibiotic-resistant bacteria. In a retrospective cohort study from Belgium, colonization surveillance was performed through routine site-specific surveillance cultures (urine, mouth, trachea and anus). Additional cultures were performed when presumed clinically relevant. ICU patients with nosocomial bacteremia caused by antibiotic resistant Gram-negative bacteria (ABR-GNB) were included in the study. Prior colonization was defined as the presence of the same ABR-GNB in colonization and subsequent blood cultures. It was also required that these strains were detected ≥ 2 d before the onset of bacteremia. It was observed that 75% of episodes of bacteremia were preceded by colonization. Appropriate empiric antibiotic therapy (started within 24 h) was administered for 74.4% of those episodes with colonization vs. 55.0% of the episodes that occurred without prior colonization. Appropriate therapy was administered within 48 h for 100% episodes preceded by colonization vs. 90.0% of episodes without prior colonization.[14]

Colonization surveillance has the potential to improve empiric antimicrobial treatment adequacy in a critical care setting. In a study from Greece, colonization surveillance of the respiratory tract and gastrointestinal tract was systematically performed in all ICU patients. Tracheal aspirates were obtained twice weekly and rectal swabs once weekly. Both tracheal and rectal samples were cultured in antibiotic-enriched media (containing ceftazidime, ciprofloxacin, imipenem or piperacillin/tazobactam), to focus on resistant Gram-negative pathogen isolation. Colonization concordance was 82% in VAP (ventilator-associated pneumonia) and 86% in BSI (blood stream infection) cases. Previous colonization had high sensitivity and specificity in VAP, but was less specific in BSI cases. Knowledge of previous colonization improved the rate of adequate empiric antimicrobial treatment (91 vs. 40% in VAP and 86 vs. 50% in BSI cases).[15]

**Antibiotic use as a risk factor for the acquisition of MDROs.** A multinational survey of risk factors (from six centers in Europe, Asia and North America) for infection with extended spectrum β-lactamase (ESBL) producing Enterobacteriaceae in non-hospitalized patients reported that recent antibiotic use, residence in a long-term care facility, recent hospitalization, age 65 y and male sex were risk factors.[16] Another study from Cameroon showed that previous use of antibiotics (ciprofloxacin) appeared to be a risk factor for ESBL carriage.[17]

**Antibiotic-resistant bacteria in food chain.** There is growing concern that antibiotic-resistant bacteria may have contaminated some part of the human food chain, resulting in subsequent colonization of human intestinal tract. A high prevalence of ESBL genes was found in chicken meat (79.8%) in the Netherlands. Genetic analysis showed that the predominant ESBL genes in chicken meat and human rectal swab specimens were identical. These genes were also frequently found in human blood culture isolates. Typing results of *Escherichia coli* strains showed a high degree of similarity with strains from meat and humans. These findings suggest that the abundant presence of ESBL genes in the food chain may have a profound effect on future treatment options for a wide range of infections caused by Gram-negative bacteria.[18]

In another Dutch study it was observed that 35% of the human isolates contained poultry associated ESBL genes and 19% contained poultry associated ESBL genes located on Inc.I1 plasmids that were genetically indistinguishable from those obtained from poultry (meat). Of these ESBL genes, 86% were bla (CTX-M-1) and bla (TEM-52) genes, which were also the predominant genes in poultry (78%) and retail chicken meat (75%). Of the retail meat samples, 94% contained ESBL-producing isolates of which 39% belonged to *E. coli* genotypes also present in human samples. These findings are suggestive for transmission of ESBL genes, plasmids and *E. coli* isolates from poultry to humans, most likely through the food chain.[3]

## Laboratory Methods for Detection of Resistant Pathogens in Surveillance Microbiology

There are two principal approaches for the detection of antibiotic resistant bacteria in surveillance microbiology samples—culture-based method and molecular method (**Table 2**).

**Surveillance culture for detection of MDROs.** *Surveillance culture of stool or rectal swabs for antibiotic-resistant bacteria.* Surveillance culture of stool is a method which could be utilized for the detection of common pathogens (which could be present as pathogen carriers, e.g., Salmonella) or for the detection of multidrug-resistant bacteria. In clinical settings where infection and colonization due to multidrug-resistant bacteria is significant clinical problem, the method could be used for early detection of MDROs (multidrug-resistant organisms). Early detection would enable early recognition of carriers, institution of appropriate infection control precautions, counseling of the patients and relatives about optimal measures to prevent infection and transmission and more informed selection of appropriate empirical antimicrobial therapy in case of infection episodes.

In the culture-based methods various samples from appropriate anatomical sites (depending on the common reservoir of resistant organisms) are inoculated onto a suitable culture media (antibiotic impregnated), incubated at a specific temperature and following growth after a definite incubation period (24–48 h) are identified by conventional phenotypic microbiological techniques (culture characteristics, Gram stain, biochemical reactions and susceptibility to antimicrobial agents). Antibiotic susceptibility confirmation can then be performed using standardized criteria established by reputed organizations such as CLSI (Clinical Laboratory Standards Institute), EUCAST (European Committee on Antimicrobial Susceptibility Testing), BSAC (British Society for Antimicrobial Chemotherapy), SRGA (Swedish Reference Groups for Antibiotics), etc. The common sample types and resistant organisms that are identifiable by this method include nose/throat/groin/axilla swab for MRSA, rectal swab/stool for VRE and MDR-GNB (multidrug-resistant Gram-negative bacilli). There is a need to have a SOP (standard operating procedure) and robust quality control to ensure accuracy and reproducibility of the results.

The criteria used to detect and identify antibiotic resistance can play a significant role on the data and its subsequent interpretation. In a Swedish study it was reported that the species-related SRGA (Swedish Reference Group for Antibiotics) breakpoints detected Gram-negative isolates with decreased susceptibility

**Table 2.** Strategies for early detection of MDROs (multidrug-resistant organisms)

| Strategy | MDRO type | Sample type | Laboratory method | Approximate TAT |
|---|---|---|---|---|
| Surveillance | MRSA | Nose swab, throat swab, groin swab, ulcer/skin lesion | Culture, PCR. Example of commercial system for PCR: BD, Cepheid | Culture: 3 d; PCR: same day |
| Surveillance | VRE | Stool, rectal swab | Culture, PCR. Example of commercial system for PCR: Cepheid | Culture: 4–5 d; PCR: same day |
| Surveillance | ESBL | Stool, rectal swab | Culture | Culture: 4–5 d; |
| Surveillance | MBL/CRKP | Stool, rectal swab | Culture; In house PCR | Culture: 4–5 d; PCR: same day |
| Diagnostic testing | Direct antibiotic susceptibility on positive blood or body fluid cultures (BSAC method) | Blood or sterile body fluid in a blood culture bottle | Blood or body fluid culture followed by direct susceptibility test on positive broth | One day after culture is flagged positive in automated system |

TAT, turnaround time; MRSA, methicillin-resistant *Staphylococcus aureus;* VRE, vancomycin resistant Enterococcus; ESBL, extended spectrum β-lactamase; MBL, metallo-β-lactamase; CRKP, carbapenem-resistant *Klebsiella pneumoniae.*

more frequently in comparison than the NCCLS (National Committee for Clinical Laboratory Standards) breakpoints. The BSAC (British Society for Antimicrobial Chemotherapy) breakpoints for susceptible organisms were similar to NCCLS for ciprofloxacin and imipenem, and similar to SRGA for ceftazidime but lower than both NCCLS and SRGA for gentamicin, causing a much higher frequency of decreased susceptibility to gentamicin.[19] These observations show the importance of standardized breakpoint interpretative criteria used in reporting of results, and the subtle differences in the breakpoint of various antibiotics in different international standards.

The sensitivity of detection of MDROs is different based on the specific culture methodology used. The media used, the antibiotic used for screening, the concentration of the antibiotic used, and the technique of inoculation can all play very important roles in identifying resistant organisms. In a study done at Tel Aviv, three agar-based methods for direct CRE (carbapenem-resistant Enterobacteriaceae) detection from rectal swabs were compared: CHROMagar-KPC (Chrom), MacConkey agar with imipenem at 1 μg/ml (MacI) and MacConkey plates with imipenem, meropenem and ertapenem disks (MacD). The levels of detection (LODs) of CRE strains were influenced by their MICs to carbapenems and were best for MacI, followed by CHROM. The MacD method was able to detect only the strains exhibiting MICs of ≥ 32 μg/ml to at least ertapenem. Both CHROM and MacI had greater sensitivity (85%) than MacD (76%). However, MacI was the most specific method. Imipenem impregnated MacConkey agar (MacI) was found to be the most appropriate medium for the detection of CRE.[20] In another study, the method of placing a sample from a fecal surveillance culture into broth containing a 10-μg imipenem disk appeared to have the greatest sensitivity for detecting KPC-producing *K. pneumoniae*. *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, which could also be resistant to carbapenem antibiotics, were detectable using this method.[21]

*Surveillance culture from respiratory samples.* There is a lack of consensus internationally (more so in resource constrained settings) about the value of surveillance cultures for antibiotic-resistant bacteria in reducing mortality and morbidity in clinical settings. However, several studies demonstrate the importance of surveillance microbiology of respiratory samples where hospital-acquired chest infection (with or without blood stream infection) was predicted by surveillance cultures. In a retrospective observational study from Ghent, pathogen prediction for blood stream infection by tracheal surveillance cultures in cases of hospital-acquired pneumonia was associated with a higher rate of adequate empiric antibiotic therapy (71% vs. 45%). Pathogen prediction was associated with increased survival in both univariate (OR 0.43; CI 0.19–0.93) and multivariate analysis (OR 0.32; CI 0.12–0.82). Multivariate analysis further identified methicillin-resistant *Staphylococcus aureus* (MRSA) (OR 5.90; CI 1.36–25.36) and *Pseudomonas aeruginosa* (OR 3.30; CI 1.04–10.4) as independent risk factors for mortality.[22] These results assumes significance because of the fact that in specific infections caused by organisms such as MRSA or Pseudomonas only certain antibiotics could be used for effective therapy. For example, for MRSA the antibiotic options include glycopeptides (vancomycin and teicoplanin), oxazolidinones (e.g., linezolid), lipopeptides (e.g., daptomycin except in case of MRSA pneumonia where there is surfactant mediated deactivation of the drug) and glycylcyclines (e.g., tigecycline). In certain cases based on the sensitivity, clinical severity and the anatomical site of the infection tetracycline (e.g., doxycycline), sulfonamides (e.g., co-trimoxazole) or MLS group antibiotics (e.g., clindamycin, erythromycin) and ripampicin (in combination therapy) are also usable. All β-lactam antibiotics (penicillins, cephalosporins and carbapenems) would be ineffective in MRSA except ceftobiprole (a novel fifth generation cephalosporin with activity against MRSA). Similarly, not all antibiotics used in the treatment of Gram-negative bacillary infections would be effective against *Pseudomonas aeruginosa*. Active agents (sensitivity-dependent) include fluoro-quinolones (e.g., ciprofloxacin and levofloxacin), ceftazidime (a third generation cephalosporin), cefepime (a fourth generation cephalosporin), aztreonam (a monobactam), piperacillin-tazobactam

(a β-lactam + β-lactamase inhibitor combination), carbapenems (e.g., imipenem and meropenem but not ertapenem) and aminoglycosides (e.g., gentamicin and amikacin). Common antibiotics with no activity against Pseudomonas include co-amoxiclav, cefuroxime, cefotaxime/ceftriaxone and tigecycline. In another study by the same group, tracheal surveillance culture predicted multiple-drug-resistant etiology of bloodstream infection associated with pneumonia in 70% of patients. In about 15% of patients discordant resistant pathogens were identified. In that study, surveillance cultures were taken thrice weekly urinary cultures and oral swabs, once weekly anal swabs and thrice weekly tracheal aspirates in intubated patients. In the subgroup of patients with two risk factors (based on length of prior intensive care unit stay and prior antibiotic exposure) for multiple-drug-resistant infection, incorporating results of surveillance cultures moderately contributed to adequacy of early antibiotic therapy (from 75% to 90%) while limiting antibiotic consumption.[23] In a different prospective observational study in a French medical ICU endotracheal aspiration (EA) performed twice weekly in all mechanically ventilated patients made it possible to prescribe adequate antibiotic therapy (while waiting for BAL culture results) in 95% of the patients in whom a VAP (ventilator-associated pneumonia) was ultimately diagnosed by BAL culture.[24]

**Molecular methods for the detection of MDROs in surveillance samples.** Surveillance culture for antibiotic resistant bacteria is time-consuming, labor-intensive and expensive for the laboratory to perform. Molecular methods for the detection of MDROs relies on the detection of specific gene targets by various molecular biology techniques of which PCR (polymerase chain reaction) is the principal and most commonly used. In this method a specific gene target that represents the signature sequence of a resistant pathogen (e.g., *SCCmec/orfX* junction in MRSA PCR, *VanA/VanB/VanC* genes in VRE PCR or *blaKPC* gene in carbapenem-resistant *Klebsiella pneumoniae*) is amplified several billion-fold in the PCR reaction mix using specific set of primers. The PCR product is detected either during amplification (in real time PCR) or post amplification (in end point PCR using agarose gel electrophoresis). This approach enables the rapid, detection of target genes which are present in low numbers in the target sample (high sensitivity). The specificity may be affected unless stringent quality control measures are not used to prevent amplicon contamination. Sensitivity could be compromised because of the presence of PCR inhibitors, especially if DNA extraction techniques are not optimized. In one study from Baltimore, the sensitivity and specificity of the commercial BD-MRSA PCR was found to be 89% and 91.7% respectively. The positive predictive value (PPV) of the MRSA PCR here was found to be 65.9%. The low PPV could be because of the presence of dead bacteria, or non-specific amplification.[25] In a study from Tel Aviv, Israel, a PCR-based surveillance test for identification of rectal carriage of KPC (*Klebsiella pneumoniae* carbapenemase)-producing CRE (carbapenem-resistant Enterobacteriaceae) specimens was evaluated by culture and by PCR analysis for *blaKPC*. Concordant results were documented in a significant majority of clinical isolates. The sensitivity and specificity of the PCR analysis were 92.2% and 99.6%, respectively, and those of the culture was 87.5% and 99.4%, respectively. The turnaround time was 30 h for the PCR and 60 h (negative) and 75 h (positive) for the CRE culture. *blaKPC* PCR-based testing was found to be sensitive and rapid method for the surveillance of KPC-producing CRE.[26]

Detection of vancomycin-resistant Enterococus represents another target after MRSA where molecular techniques based on PCR are being used more often. In a CDC (Center for Disease Control and Prevention) study PCR primers directed to *vanA*, *vanB*, *vanC1*, *vanC2* and *enterococcal ligase* genes were used to detect and identify VRE in fecal material obtained by rectal or perirectal swabbing. Although PCR-inhibitory substances were present in DNA prepared directly from the swabs (a major concern that may give rise to false negative PCRs), the inhibitory substances could be reduced by processing the nucleic acid with two commercially available DNA preparation columns. Testing and retesting strategies need to be clearly defined in any PCR standard operating procedure. It was observed that by using all four primer sets, only 67.8% of the samples were positive for vanA. However, after retesting the negative samples were positive. The specificity of the vanA assay was 99.6%. The sensitivity of the PCR could be increased by enrichment culture techniques. For example, PCR analysis of enrichment broth samples after 15 to 18 h of incubation detected 85.1% of culture-positive specimens. The specificity of the vanA assay after the enrichment step was 100%. PCR-based detection allowed the identification of VRE with a turnaround time of 8 h. The cost of the test was $10.12 per assay. It can be concluded from this study that PCR may be a cost-effective alternative to culture for surveillance of VRE in some hospitals especially if we take into account the clinical and infection control benefits of early detection of MDROs.[27]

**Early diagnosis of infections caused by MDROs.** *Direct susceptibility testing from positive blood cultures as a method for early detection of resistant bacteria.* It takes about 48 h (or two working days) to get the antibiotic susceptibility of organisms isolated from positive blood cultures. This is because of the time required to isolate colonies and perform antibiotic susceptibility tests (ASTs) from them. This delay in getting ASTs by the conventional methods can be critical in seriously ill patients. The direct susceptibility testing (DST) from positive blood culture broths is an attempt to overcome this problem. The DST is performed as per recommended guidelines (e.g., British Society for Antimicrobial Chemotherapy) based on Gram stain findings from positive blood culture broths. The advantage of this method include (1) reduction in TAT (turnaround time) and (2) clones of bacteria identical in colony morphology and biochemical characteristic but different in antibiotic susceptibility can be represented in the final report. The disadvantages include (1) DST results need to be confirmed by conventional methods and (2) DST cannot be done or results not reliable in mixed infections (Gram-positive and Gram-negative) or infections caused by yeasts (e.g., *Candida* species) where inoculum preparation standards may not be available.

The essential agreement (sensitive or resistant isolates interpreted as intermediate or vice-versa), and the categorical agreement (absolute agreement in sensitive/intermediate/resistant

categories), minor error (total number minus essential agreement), major errors (isolate reported resistant when it is actually sensitive), very major error (isolate reported sensitive when it is actually resistant) have been studied and reported in various studies. For example, Chapin and Musgnug reported 98% essential agreement and categorical error rates of 0.3% minor, no major (false resistance) and 1.7% very major (false susceptibility) errors in Gram-positive isolates, and 99% essential agreement, 0.5, and 0 and 2.0% respectively for minor, major and very major errors, respectively.[28] The reporting time for the direct testing of susceptibility for blood culture isolates by the VITEK 2 system ranged from 3.3 to 17.5 h. Compared with conventional methods that require 1 or 2 d, this method can make same-day reporting possible and thus permit better patient management.[29] In a study done by Chen et al. 89.7% of Gram-negative bacilli and 33.3% of Gram-positive cocci were correctly identified to the species level. For antimicrobial susceptibility testing, the direct method had an overall error rate of 5.4% for Gram-negative bacilli, with 0.9% very major, 0.9% major and 3.6% minor discrepancies compared with the standard method. The overall error rate in antimicrobial susceptibility testing for the 13 *Staphylococcus* spp. was 10.3%, with 6.0% very major, 2.6% major and 1.7% minor discrepancies.[30] In a study done by Coyle et al. the time of reading of DST based antibiotic susceptibility was found to have an effect on the degree of agreement. When minor discrepancies were ignored, the 4 h readings were in agreement for 98% of the tests with Gram-positive organisms and 95% of the tests with Gram-negative organisms. After 6 h of incubation, 91% of the tests with Gram-positive organisms and 86% of the tests with Gram-negative organisms agreed with standard results. The frequencies of major discrepancies were 3.5% after 4 h, 0.6% after 6 h and 0.7% after overnight incubation.[31] Edelmann et al. found that with regard to Gram-positive cocci agreement between DST and conventional method was found in 93.9% of cases, with 1.6% very major, 1.1% major and 2.6% minor errors. For Gram-negative rods agreement was found in 91.9% of cases, with 1.2% very major, 0.7% major and 6.3% minor errors. The standards used in interpretation of breakpoints, and specific bacteria-antibiotic combinations can also affect the degree of agreement. When applying the breakpoints of the Deutsches Institut für Normung for interpretation of MICRONAUT tests, agreement of direct disk diffusion with standard testing decreased to 82.4% in Gram-negative rods, with 3.6% very major, 0.5% major and 13.4% minor errors. A high rate of disagreement was observed with oxacillin and gentamicin in Gram-positive cocci, and with cefuroxime, amoxycillin/clavulanate and piperacillin/ tazobactam in Gram-negative rods.[32]

*The role of new rapid molecular tests in the detection of MDROs in the diagnostic setting.* One of the biggest draw backs of the conventional culture-based methods has been the delay of 24–48 h (sometimes more in case of mixed cultures and slow growing organisms) even after automated blood culture systems such as the Bactec or BacT/ALERT had flagged a blood culture positive. Therefore, there is a need for faster diagnostic methods so that treatment could be optimised especially in the case of infections caused by multidrug-resistant organisms. Molecular methods based on polymerase chain reaction and micro-arrays (gene chips) are being studied with the objective of developing faster, accurate diagnosis with more detailed information. Palka-Santini et al. reported from Germany the development a microarray for the identification of *Staphylococcus aureus*, which could reduce the blood culture post-processing time to a single day. The technique allowed concomitant identification of virulence factors and antibiotic resistance determinants directly from positive blood cultures. The assay identified most of the important virulence genes such as tsst-1, sea, seb, eta and antibiotic resistance genes such as mecA, aacA-aphD, blaZ and ermA.[33] Similarly, Fujita et al. evaluated the usefulness of PCR analysis followed by microchip gel electrophoresis (MGE) of the 16S-23S rRNA gene internal transcribed spacer (ITS) or the CTX-M gene for direct identification of Gram-negative bacteria (GNB) from positive blood culture bottles. In the ITS-based method 90% of blood cultures were correctly identified. In the method based on the PCR of bla(CTX-M), bla(SHV) and bla(TEM) genes of 109 ESBL-producing isolates from various clinical materials CTX-M ESBL was detected in 105 isolates, and SHV ESBL was detected in two isolates. There was also a high degree of correlation between combination disk method (for phenotypic detection of ESBL), and PCR-MGE (microchip gel electrophoresis) method. The molecular method-based results were obtained within 1.5 h at a calculated cost of $6.50 per specimen.[34]

Cleven et al. from Germany reported identification and characterization of bacterial pathogens causing bloodstream infections by DNA microarray. The array consisted of 120 species-specific gene probes (for example, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*) 200 to 800 bp in length. These probes represented genes encoding housekeeping proteins, virulence factors and antibiotic resistance determinants. Evaluation revealed that the DNA microarray was highly specific in identifying *S. aureus, E. coli* and *P. aeruginosa* strains and in discriminating them from closely related Gram-positive and Gram-negative bacterial strains. They found a nearly perfect correlation between phenotypic antibiotic resistance determined by conventional susceptibility testing and genotypic antibiotic resistance by hybridization to the *S. aureus* resistance gene probes *mecA* (oxacillin-methicillin resistance), *aacA-aphD* (gentamicin resistance), *ermA* (erythromycin resistance) and *blaZ* (penicillin resistance) and the *E. coli* resistance gene probes blaTEM-106 (penicillin resistance) and aacC2 (aminoglycoside resistance).[35]

Naas et al. evaluated the utility of DNA microarray, using the check-points ESBL/KPC array for rapid detection of TEM, SHV and CTX-M extended-spectrum β-lactamases and KPC carbapenemases. It was observed that the Check-Points ESBL/KPC array (a commercial system), allowed faster detection (along with high throughput) of all TEM, SHV and CTX-M ESBL genes and of the KPC-2 gene from culture isolates. The assay allowed easy differentiation between non-ESBL TEM and SHV and their ESBL derivatives and had high specificity. The technique was found suitable for Enterobacteriaceae and non-fermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Results were available within the same working day, allowing rapid implementation of isolation measures and appropriate antibiotic treatment.[36] The same group of investigators reported a

sensitivity and specificity of 100% with the Check-MDR CT102 microarray, aimed at identifying bacteria producing extended spectrum β-lactamase (ESBL) (SHV, TEM and CTX-M) and carbapenemase (KPC, OXA-48, VIM, IMP and NDM-1), with 144 Gram-negative strains.[37] Another group of investigators from the Netherlands reported a sensitivity of 97%, with 100% specificity while testing the Check-MDR CT102 DNA microarray on 70 carbapenemase-producing isolates with meropenem MICs ≥ 0.5 mg/L. For ESBL detection, the sensitivity was 100% and the specificity was 98%.[38] Fishbain et al. from the USA reported a high degree of concordance (90% to 98.3%) between nucleic acid microarray and phenotypic results. Compared with phenotypic testing, the sensitivity and specificity of the microarray was between 88.9% and 94.4% and 100% respectively.[39]

Rapid molecular methods could also help in confirming or correcting results obtained by phenotypic methods. Wintermans and colleagues showed the limitations of phenotypic conventional tests in the detection of antibiotic resistant bacteria. They looked at all patients that had been in isolation because of the detection of ESBL according to the ESBL E-test (a phenotypic method). When strains were retested using a genotypic method (a commercially available Check-Point microarray) 14% patients ESBL carriage could not be confirmed with the microarray. This was verified with PCR and sequencing. False-positive results from phenotypic tests resulted in a total of 151–279 d of unnecessary isolation.[40]

The microarrays have been used for the detection of virulence and epidemiological markers besides antibiotic resistance gene. Spence et al. reported the validation of virulence and epidemiology DNA microarray for identification and characterization of *Staphylococcus aureus* isolates. The array comprised 84 gene targets, including species-specific, antibiotic resistance, toxin and other virulence-associated genes, and which was capable of examining 13 different isolates simultaneously, together with a reference control strain. The study described the ability of the array to differentiate between isolates representative of a spectrum of *S. aureus* types, including methicillin-susceptible, methicillin-resistant, community-acquired and vancomycin-resistant *S. aureus*, and to simultaneously detect clinically relevant virulence determinants.[41]

However molecular assays are some distance away from replacing culture based methods for diagnosis of infections. Pammi et al. in 2011 in a meta-analysis of the 23 studies on the molecular assays in the diagnosis of neonatal sepsis found the mean sensitivity and specificity to be 0.90 (95% CI: 0.78–0.95) and 0.96 (95% CI: 0.94–0.97), respectively. Real-time polymerase chain reaction (PCR) and broad-range conventional PCR had higher sensitivity and specificity than other assays. If optimal sensitivity and specificity of molecular assays in comparison to the reference method (microbial culture) were taken as 98% and 95% respectively then according to the meta-analysis molecular assays did not have sufficient accuracy to replace culture based methods. The molecular assays may however perform well as "add-on" tests.[42] The other drawback of molecular methods of resistance detection (apart from cost and initial capital investment required) has been the reliance on detection of known resistance cassettes. This technique is intrinsically vulnerable due to the inability to detect newly emergent resistance genes. Waldeisen et al. reported the development of a real-time PCR antibiogram for drug-resistant sepsis which precluded the problem. The investigators describes a real-time PCR procedure [with high sensitivity (< 100 CFU/mL)] that determines susceptibility by monitoring pathogenic load with the highly conserved 16S rRNA gene in blood samples exposed to different antimicrobial drugs. After real-time PCR-based determination of pathogenic load, a $\Delta C(t) < 3.0$ between untreated and treated samples was found to indicate antimicrobial resistance. Species identification was performed via analysis of the hypervariable amplicons. The real-time PCR was able to give reports (which included identification and susceptibility testing) in less than 24 h.[43]

Although the importance of PCR based molecular assays in the detection of MDROs is being increasingly recognized, there are few studies on the role of quantitative real time PCRs to measure the bacterial load and correlate it with prognosis. In a study in Taiwan, a quantitative real-time PCR assay for the mecA gene evaluated the impact of bacterial load on prognosis. Known copy numbers of a plasmid containing mecA DNA were used as a standard and the previously described mecA-specific primers and probe were used. It was observed that the levels of mecA DNA in the non-survivors were significantly higher than those in the survivors. Moreover, the non-survivors had higher mecA DNA levels than the survivors after 3 d and 7 d of anti-MRSA therapy (median mecA DNA copy numbers for non-survivors and survivors at 3 d, 5.86 and 4.30 log copies/ml, respectively; whereas, medians for non-survivors and survivors at 7 d, 5.21 and 4.36 log copies/ml, respectively). Together, these findings suggest that the level of mecA DNA in blood could potentially be used to monitor MRSA bacteremia and evaluate responses to therapy.[44] A similar study is in the process of recruiting patients at Taiwan to establish a quantitative PCR for VRE bacteremia.[45]

## Re-Infection, Recurrence, Re-Colonization, Persistence with MDROs

MDROs, especially the ones which colonize the gut (e.g., ESBL, VRE and carbapenem-resistant Enterobacteriaceae) may persist in the gut for an indefinite period of time. There are also limitations in the sensitivity of culture-based surveillance. This makes infection control practicalities such as barrier nursing, cohorting or isolation precautions challenging. There are also no effective antimicrobial agents available to ensure decolonization of gut colonized patients. Re-infection, recurrence and re-colonization are possibilities.

It has been noted that antibiotic therapy may be associated with recurrent VRE stool colonization in many patients who have previously had three consecutive negative stool cultures. It has been suggested that these patients should be screened for recurrent stool colonization when antibiotic therapy is administered. In one study, 62% of patients who received antibiotics, previously known to be negative for VRE, developed recurrent high-density VRE stool colonization during a course of therapy. PFGE analysis showed that recurrent strains were unrelated to

**Table 3.** Cost of intravenous antimicrobial therapy

| Antibiotic | Adult daily dose | Daily cost of therapy (adult) |
|---|---|---|
| Piperacillin-tazobactam | 4.5 g IV TID | Rs. 2,028 to Rs. 2,880 ($37 to $52) |
| Meropenem | 1 g IV TID | Rs. 2,697 to Rs. 7,488 ($49 to $136) |
| Colistin | 2 MU IV TID | Rs. 3,000 to Rs. 5,670 ($55 to $103) |
| Tigecycline | 100 mg IV loading dose, then 50 mg IV BID | Rs. 5,600 to Rs. 5,980 ($102 to $109) |
| Linezolid | 600 mg BID | Rs. 2,249 ($41) |
| Teicoplanin | 400 mg IV BID loading dose, then 400 mg IV OD | Rs. 1,534 to Rs. 1,760 ($28 to $32) |
| Daptomycin | 6 mg/kg IV OD | Rs. 4,804 ($87) |

Prices are based on maximum retail prices of commercial brands in India. Conversion to US Dollar prices was based on rates in August 2012.

**Table 4.** Economic figures with respect to the management of patients with MDROs in an Indian hospital

| Economic parameter | Figure |
|---|---|
| Poverty line in India | Daily income of less than or equal to Rs. 28 ($0.50) per day |
| Gross National Income per capita in India | $1,410 per year or $117 per month (2011 data, World Bank) |
| Private ward hospital charges (B- class) | Rs. 1,100 ($20) per day (AIIMS, India) |
| Intensive Care bed charges | Rs. 2,100 (Rs. 1,000) or $38/day over the bed charges per day (AIIMS, India) |
| Daily cost of Meropenem therapy in an adult patient | $49 to $136 (based on MRP of some brands available in India) |
| Stool culture | Rs. 50 or ~$1 (AIIMS, India) |
| Antibiotic sensitivity test | Rs. 50 or ~$1 (AIIMS, India) |
| PCR test (minimum rate e.g., qualitative test for CMV, HSV) | Rs. 600 or ~$12 (AIIMS, India) |

the prior strain in 3/5 patients, closely related in 1/5 patients and indistinguishable in 1/5 patients.[46] Colonization with VRE may persist for years, even if the results of inter-current surveillance stool and index site cultures are negative. Recurrence may occur due to antibiotic therapy.[46] Cultures for detection of VRE in stool samples obtained from patients declared "cleared" were found to be insensitive.[47] In a prospective cohort study in Boston, VRE colonization at the time of ICU (intensive care unit) admission was associated with the use of second- and third-generation cephalosporins [odds ratio (OR) = 6.0], length of stay prior to surgical ICU admission (OR = 1.06), greater than 1 prior ICU stay (OR = 9.6) and a history of solid-organ transplantation (OR = 3.8). It was concluded in that study that exposure to second- and third-generation cephalosporins, was an independent risk factor for colonization.[48]

## Economic Issues in the Management of Infections Caused by Multidrug-Resistant Organisms

Infections due to multidrug-resistant organisms (MDROs) are often difficult to treat. For many patients infected with MDROs there are no effective orally active antibiotics, thereby increasing the cost of management. In complicated systemic infections involving vital organ systems, patients have to be treated in hospitals and sometimes in critical care units. Sometimes because of the difficulty and delay in diagnosis additional resources are required over the baseline cost in the treatment of drug-sensitive organisms. In economic models of healthcare where a significant burden of cost may have to be borne by out of pocket expenditure by patients and relatives, the cost of management of infections due to MDROs could be economically crippling. In countries where the state totally or partially pays or subsidizes the cost of treatment the additional resource required for detection, treatment and infection control is significant. The cost of antimicrobial agents constitutes the major share in this additional resource requirement. Economic figures from a developing country like India, shows the gross disparity in some cases between the cost of antimicrobial therapy and the average income of the general population (**Tables 3 and 4**).[49-51]

Colonization of individuals with drug-resistant organisms may subsequently lead to infections with these agents. There lies the justification of many screening programs. The association between colonization rates and serious infection such as blood stream infections (BSIs) has been investigated. A study from Chicago showed that an increase in colonization with ESBL-producing Enterobacteriaceae from (1.33% in 2000 to 3.21% in 2005) was accompanied by an increase in ESBL-positive blood stream infection (4-fold rise in the same period). About 8.5% of those colonized with ESBL developed blood stream infection and about half of those who developed BSI were not previously screened for ESBL.[52]

The utility of universal screening has been a contentious issue and the benefits in some cases may be marginal or not at all. It is likely that screening would lead to an increase in the detection rates of colonized patients, but this may not translate into reduction of infection rates. A Wisconsin study on universal screening for MRSA by PCR was associated with a modest increase in MRSA detection of 2.95%, and a non-significant decline in hospital-acquired MRSA infections by 0.12%. The benefit-to-cost ratio was 0.50, suggesting that for every dollar spent on universal vs. targeted screening, only $0.50 was recovered in avoided costs of hospital-acquired MRSA infection.[53] The benefit and the

cost-effectiveness of the screening program could also be dependent on the prevalence of the condition being screened. Studies by the UK-based Health Protection Unit using a theoretical population of patients in an intensive care unit using dynamic transmission model indicated that universal admission screening and weekly screening for MRSA by polymerase chain reaction combined with decolonization would be cost effective only if the prevalence was at least 10%.[54] In contrast there are reports from the US and Europe to suggest that PCR testing for MRSA would be cost-effective across a wide range of MRSA prevalence rates and PCR test costs. It was reported that the mean mortality rates were 23% for patients receiving empiric vancomycin subsequently switched to semi-synthetic penicillin (SSP) for MSSA, 36% for patients receiving empiric vancomycin treatment for MRSA, 59% for patients receiving empiric SSP subsequently switched to vancomycin for MRSA and 12% for patients receiving empiric SSP for MSSA. The numbers of patients needed to test in order to save one life were 14 and 16 if the MRSA prevalence was 30%, compared with empiric vancomycin and SSP, respectively. In the EU, the cost-effectiveness ratios for empiric vancomycin- and SSP-treated patients were €695 and €687 per life-year saved, respectively, compared with €636 per life-year saved for rapid PCR testing. In the US, the cost-effectiveness ratio was $898 per life-year saved for empiric vancomycin and $820 per life-year saved for rapid PCR testing.[55]

The policy of using culture-based assays or molecular assays for screening patients for resistant organisms would depend on the prevalence of the organism of interest and cost of the technology used. For example, the costs of screening and isolation per averted MRSA infection was estimated to be lowest using selective chromogenic culture-based screening in high (15%) and medium (5%) prevalence settings, at $4,100 and $10,300, respectively. Replacing the chromogenic culture-based test with a PCR-based test would cost $13,000 and $36,200 per additional infection averted.[56]

While doing the cost benefit analysis the hidden cost associated with detection of resistant pathogens is not always evaluated or appreciated. For example, the status of the patient with regard to the colonization or infection with multidrug-resistant pathogens could have a significant impact on ward management of patients, especially while taking into consideration the limitations in availability of isolation bed. In a survey of patient access managers (PAM) on the impact of contact precautions (CP) for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE) on time to bed assignment it was found that the vast majority (> 90%) of responding PAMs reported spending substantially more time assigning inpatient beds to patients who required CP accommodations for MRSA/VRE, with estimates of a doubling of the time required for other patients.[57]

## Research Questions

Infections caused by MDR-GNBs (multidrug-resistant Gram-negative bacilli) are an important cause of morbidity, mortality, increased hospital stay and health care cost. In view of the global public health significance of MDR-GNBs and several unresolved questions there is need of more research. Several authorities have already stressed on the need of integration of basic, clinical and translational research. Some recent investigations in the field of whole genome sequencing, and gene expression profiling, have made great strides in revealing the pathogenesis of infections caused by multidrug-resistant organisms, and are contributing in the development of better diagnostic tools, and prognostic markers for the management of such infections. The relevant research questions may be summarized as follows:

**Research questions with regard to diagnosis of MDR-GNBs.** (1) What is the optimal method for detection of MDR Gram-negative bacilli? Culture based approach or approach based on molecular methods?

(2) What is the optimal frequency of screening for multidrug-resistant Gram-negative bacilli (MDR-GNB) in outpatient and inpatient settings?

(3) Is it cost-effective to screen for MDR-GNB?

**Research questions with regard to treatment of MDR-GNBs.** (4) Is surveillance culture-based antimicrobial treatment planning superior to an approach based on empirical use of broad spectrum antibiotics based on the epidemiology of MDR-GNB in a population?

(5) Identification of novel antimicrobial agents that could be used in decolonization of individuals colonized with MDR-GNB.

(6) Identification of novel anti-microbial agents for the treatment of MDR-GNBs.

**Research questions with regard to pathogenesis of MDR-GNBs.** (7) Is the virulence of MDR-GNBs different to those of sensitive strains?

(8) What is the role of genomics and whole genome sequencing of bacteria in revealing:[58-65]

- The pathogenesis of these infections?
- Identification of the prognostics markers?
- The host pathogen interaction in infection and colonization?
- Identification of resistant determinants.

**Research questions with regard to infection control of MDR-GNBs.** (9) Development of more user-friendly options to increase hand hygiene adherence of health care workers.

(10) What are the reservoirs of MDR-GNBs in the environment?

## Conclusion

With the widespread dissemination of multidrug-resistant (MDR) bacteria globally there is a need of global as well as local evidence based guidelines to optimize the management of patients infected or colonized with these bacteria. In MDR bacteria, where definitive decolonization and treatment options exist (e.g., methicillin-resistant *Staphylococcus aureus* or MRSA) there is evidence that targeted screening in high risk situations is cost effective and may help not only in infection prevention efforts but also in taking appropriate decisions regarding anti-microbial therapy. However, the situation is much more complicated and contentious with regard to other organisms (e.g., multidrug-resistant Gram-negative bacilli, and glycopeptide-resistant Enterococci) where screening methods are yet to be standardized,

decolonization regimens are non-existent, duration of carriage is indefinite or unpredictable and therapeutic options in case of life threatening infections are severely restricted. There is emerging clinical evidence that early detection of resistant pathogens including those due to non-MRSA MDROs aides in early institution of appropriate anti-microbial therapy thereby reducing morbidity and mortality.

Last but not the least, many of the antimicrobial agents used on the treatment of multidrug-resistant organisms are expensive pharmaceutical products. The cost of therapy assumes special significance in the context of developing countries where the cost of the medicine may sometimes be more than the daily earning of the patient or their family. In many cases treatment for poor patients is highly subsidized by government, and generic preparations of antimicrobial agents may have to be used due to resource constraints.[49-51] There have been some concerns whether the generic products are of therapeutically equivalent, even if they may demonstrate in vitro pharmacologic equivalence.[66-69] However, it must be acknowledged that in resource-poor settings where availability of expensive medicines are limited, the treating physician is left with very few options.

The presence of antibiotic resistance genes on mobile genetic elements such as plasmids, the widespread dissemination of carbapenem-resistant Gram-negative bacilli in areas with high population, presence of antibiotic resistant bacteria in environmental reservoirs have made the task of controlling antibiotic resistance extremely difficult.[70-73] Controlling the danger posed by antibiotic resistant bacteria is going to be one of the toughest challenges faced by humanity. It would require a long-term multi-pronged strategy that should include: (1) rational use of all anti-microbial drugs especially broad spectrum agents, (2) provision to the population of safe food and water which is uncontaminated with antibiotic resistant bacteria, (3) control of antibiotic use in food animals and veterinary industry, (4) restriction of over the counter prescribing of antibiotics (especially in developing countries), (5) screening and surveillance programs in the hospital for early detection of patients infected or colonized with resistant organisms and (6) adequate infection prevention and control systems which includes (but not limited to) good hand hygiene, contact precautions, good housekeeping and environmental cleaning. The high technology of micro-arrays and whole genome sequencing has to be integrated with simple measures such as cleaning, hand washing and discipline in antibiotic prescribing if we are to be anywhere near our goal of providing a better world for future generations.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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