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RISK FACTORS FOR INFECTION WITH MULTIDRUG-RESISTANT ORGANISMS IN HARYANA, INDIA

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Abstract

Objective—To investigate risk factors for multi-drug resistant organism (MDRO) infection within a hospital population in northern India.

Design—This case-control study examined MDRO infection risk factors including diet, health history and medical device use. We administered a diet questionnaire to collect data on usual diet and collected data on other risk factors from chart review.

Setting—Patients were recruited from a tertiary care hospital in northern India

Patients—All participants were inpatients identified through hospital microbiology reports. A total of 39 MDRO-patient cases and 91 controls were included.

Methods—Descriptive statistics, univariate analysis, and multivariate logistic regression were performed to evaluate the association between risk factors and MDRO infection.

Results—All cases had gram-negative MDRO infections. Univariate analyses found length of hospital stay, connective tissue disease, hospitalization in the last 12 months, hospitalization of a family member, in-hospital antibiotic use, antibiotic use in the last 12 months, and feeding tube, central venous line, and urinary catheter use to be significantly different between cases and controls. Logistic regression showed a more than three-fold increase in the odds of infection with antibiotic use in the last twelve months (OR 3.30, 95% CI 1.22–8.91) and urinary catheter use (OR

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3.63, 95% CI 1.14–11.58). Differences in dietary preferences and fruit, vegetable, and fiber consumption were not significantly associated with infection.

Conclusions—Antibiotic use is a major driver of MDRO infections. Our findings suggest that interventions optimizing antibiotic stewardship and reducing device use should be a priority to prevent MDRO infections.

Introduction

Antibiotic resistance is a major threat to human health across the globe and is rapidly becoming more common.¹ Multidrug resistant organisms (MDROs) causing infections have been rising over the past few decades worldwide. The high prevalence of MDROs such as vancomycin-resistant *Enterococcus* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), resistant gram-negative bacteria, and *Clostridium difficile* (*C. difficile*), has had a dramatic and widespread effect on human health. Preventing these infections is a major priority of international and national health agencies.

The Center for Disease Dynamics, Economics and Policy (CDDEP) reported in their “2015 Report on the State of the World’s Antibiotics” that the incidence of infections from MDROs, such as MRSA, is rapidly rising worldwide.² The burden of antimicrobial resistance in low-income countries is several times higher than in high income nations.² In India, this is a particularly prevalent problem. Many infants in the country are now born with infections resistant to most known types of antibiotics.³ MDRO infections among this vulnerable population has greatly increased infant mortality rates, so that nearly one-third of the world’s newborn deaths occur in India each year.⁴ While there is significant research regarding antibiotic resistance and the impact of MDROs in high-income countries, research on the causes, determinants, and solutions for MDROs in low- and middle-income countries such as India, is limited.

Understanding the pathogenesis and risk factors for acquisition of MDROs in these populations is important for devising context specific strategies for prevention. The niche for many MDROs is the gastrointestinal tract. There is an elaborate and complex ecosystem of anaerobic bacteria within the human gastrointestinal system. The survival and composition of this bacterial community depends many factors, including perinatal factors, diet, age, sex, and antibiotic exposure.^{5–7} In healthy individuals, mutualistic gastrointestinal microbes can provide an effective defense against invading pathogenic bacteria, including MDROs.^{8,9} However, if an imbalance in the microbial composition occurs, individuals can become more vulnerable to acquiring MDROs. Disruption of the microbiome can subsequently lead to both acute and chronic disease states.

Other risk factors for MDROs include advanced age, chronic illness, severe illness, hospital or healthcare exposure, immune suppression, nutritional factors, antibiotic use, gastric acid status, and use of devices such as endotracheal tubes, intravenous catheters, urinary catheters, and feeding tubes.^{10–15} While there are many factors that contribute to the striking rise of antibiotic resistance, the regional diet and the food sources that contribute to the diet may also have an impact on the growth of MDROs.^{16,17} The purpose of this study is to

examine prevalence of and risk factors for infection with an MDRO in inpatients at a hospital in Haryana, India, including assessment of the north Indian diet.

Methods

Research Design and Methods

The study was conducted from June to July 2016 at a 1250-bed tertiary care hospital serving a large population from the surrounding four states including Jammu & Kashmir, Punjab, Uttar Pradesh, and Bihar in northern India. Study cases were identified with the assistance of the hospital's microbiology department. They included inpatients with an MDRO infection, defined as having one or more of the following identified in clinical culture from blood, urine, sputum, or a wound: VRE, *C. difficile*, MRSA, or gram-negative bacteria resistant to three or more classes of antibiotics.

The hospital's microbiology department compiled a daily list of hospitalized patients with cultures positive for MDROs. We assessed each patient for eligibility based on inclusion and exclusion criteria. All hospital inpatients at least 18 years of age were initially eligible to participate. Exclusion criteria included: inability to communicate in Hindi or English, housed in the inpatient rehabilitation or psychiatric facilities, inability to respond (i.e. in a comatose state), altered mental state, or current use of a nasogastric tube at the time of interview. The hospital's dietetics department helped the research team provide information about the study to eligible potential participants. We aimed to recruit at least two controls for each case participant. Controls were identified by random selection from inpatient units where patients infected with MDROs were located. The same inclusion and exclusion criteria were applied to cases and controls. Records of the control participants were cross-referenced with microbiology results to ensure that they did not have any MDRO positive cultures.

We collected demographic data through chart review. Other factors with the potential to influence MDRO infection were also collected, including: body mass measures, length of hospital stay, medical and surgical history, personal and family prior hospital exposure, current and prior antibiotic and other medication use, prior MDRO infection, need for intensive care unit level care, and the presence of urinary catheters, central lines, or feeding tubes.

We used a diet questionnaire to assess daily food intake and eating habits of participants over the previous twelve months. The tool was a modified version of a validated diet instrument for use in and around New Delhi, India.¹⁸ The study hospital is geographically close to New Delhi and the cities are connected by a shared public transport subway system. The tool was reviewed and adapted with the help of the dietetics department at the study hospital. Each subject answered the questionnaire once. The questionnaire took approximately thirty minutes to complete and included questions about the types of food available to participants and how often (per day, per week, per month) they eat each food item. We separately asked participants if they were committed to a special diet.

The University of Wisconsin-Madison and study hospital Institutional Review Boards for human subjects research approved all protocols. Participants provided informed consent prior to study participation.

Statistical Analysis

Descriptive statistics and univariate analyses were performed in STATA v.14 (College Station, TX, USA). Multivariate logistic regression analysis was conducted using SAS v. 9.4 (Carry, NC, USA) with MDRO infection as the dependent variable and several potential MDRO risk factors as independent variables. The nutritional content of foods eaten by participants was calculated using the Indian Council of Medical Research¹⁹ & the USDA National Nutrient Database for Standard Reference.²⁰

Consumption of dietary fiber, fruit, and vegetables were modeled as both linear trends and in quartiles to account for any non-linear effects. Other independent variables were included in the initial model because they were consistent with our underlying conceptual model (age, sex, vegetarianism, and Charlson comorbidity index) or because they were suggestive of an association with MDRO status in univariate analysis (length of hospital stay, connective tissue disease, having a family member in the hospital, antibiotic use in the past twelve months, use of a feeding tube, use of a central venous line, and use of a urinary catheter). The final models were fit using stepwise selection with p-value cutoff of 0.2. P-values less than or equal to 0.05 and 95% confidence intervals of odds ratios that do not cross 1.0 were considered statistically significant.

Results

The final study population included 40 cases and 91 control participants. The most common site of a positive MDRO culture was urine, followed by wound and blood (Table 1). All but one of the cultured bacteria were in the MDRO group of resistant gram-negative bacilli, with the most prevalent being *Klebsiella pneumoniae* spp *pneumoniae* (Table 2). Of these gram-negative bacillus isolates, twenty-four were in the family *Enterobacteriaceae* and of those, twenty had documented resistance to carbapenems. There was one case with VRE and no cases of MRSA or *C. difficile* positive cultures.

A majority (63%) of participants were male and the median age (interquartile range; IQR) was 57 (42, 65) (Table 3). There were no significant differences in intensive care unit admissions or mean Charlson comorbidity index between groups. The average length of hospital stay was significantly longer in cases than controls (7 days vs 4 days, $p<0.001$). Cases were also more likely to have been hospitalized in the previous 12 months (63% vs 38%, $p=0.006$) and to have a family member who had been hospitalized (23% vs 11%, $p=0.047$). (Table 3). Use of antibiotics during the current hospitalization occurred in 85% of cases compared to 64% of controls ($p=0.009$). The use of antibiotics in the previous 12 months was also more common among cases, occurring in 45% of cases compared to 28% of controls ($p=0.016$).

The type of antibiotic used during hospitalizations varied widely. While no statistical comparisons were made, it is notable that colistin was used in 7 (18%) cases and no

controls. In addition, the broad spectrum antibiotic cefoperazone/sulbactam was used in 11 (28%) of cases and in 11 (12%) of controls and meropenem or imipenem was used in 10 (25%) of cases and 6 (7%) of controls. Twenty-two (55%) of cases had gram-negative isolates that were resistant to carbapenems and 3 cases had a culture positive for a gram-negative bacteria resistant to all drugs tested, including colistin.

Of the 40 MDRO cases, five had a positive culture after less than three days of hospitalization, suggesting a community onset infection. The use of clinical equipment, such as a feeding tube, central venous line, or urinary catheter, was significantly more common among cases than controls by 8%, 19%, and 32%, respectively. Estimated average annual fiber, fruit, and vegetable consumption was higher in controls than cases, however the difference was not statistically significant (Table 4). Unadjusted analysis showed that cases had a higher, but not statistically significant, prevalence of strict vegetarian diet, however vegetarianism is typically associated with being older and female, both of which were more prevalent in cases than controls.

Logistic regression models adjusting for significant confounders showed that antibiotic use in the previous 12 months and urinary catheter use were both associated with a 3-fold increase in odds of MDRO infection (Table 5). MDRO infection was not significantly associated with consumption of fiber, fruit, or vegetables, or vegetarianism in this study population.

Discussion

Multidrug resistant Gram negative bacteria are a major issue in India and worldwide.²¹ In order to combat this public health threat, risk factors for acquisition and infection need to be identified to devise effective methods of prevention and control. In our study at one medical center in India where infection with multi-drug resistant gram-negative bacteria and the use of broad-spectrum antibiotics are common, we found that the major risk factors for infection were the use of antibiotics in the prior twelve months and the use of a urinary catheter.

Prior antibiotic use is a known risk factor for MDRO infection,^{22,23} and our findings confirm this in a resource-limited setting. Most of the cases received antibiotics as expected, but a majority of control patients did as well. We confirmed that control patients did not have a positive culture, but nonetheless many received antibiotics empirically. Thus, efforts to promote antibiotic stewardship are important.

In addition to focusing on improving inpatient antibiotic stewardship, it is important to consider the outpatient setting as well. Antibiotics are widely available and used in the community setting in India without the requirement for a prescription, which increases the risk for resistance over time.^{24–27} In addition, frequent use of broad-spectrum antibiotics increases the risk for the acquisition and spread of opportunistic pathogens such as *C. difficile*.²³

We found use of a urinary catheter to be an important risk factor. Given that all the cases had gram-negative infections from a urinary source, this is not surprising. However, it suggests that interventions to minimize invasive urinary device use are likely to have a major impact,

at relatively little cost and should be pursued. This would also have the potential to improve antibiotic stewardship by reducing catheter-associated urinary tract infections and asymptomatic bacteriuria.²⁸

It is notable that we did not find patients infected with *C. difficile* or MRSA during the study period. The reasons for this are unknown, but considerable geographic diversity exists among infectious pathogens including MRSA and *C. difficile*.²⁹

We did not find dietary factors to be associated with MDRO infections in this study. However, in other studies dietary factors such as fiber have been found to promote gut microbial diversity, which in turn may decrease the risk for colonization and infection with pathogenic bacteria.^{30,31} Future studies should explore dietary factors that may impact risk of infection with gut-associated MDROs in particular.

There are several limitations in our study. Our sample size was limited by the MDRO incidence at the study hospital, although we added two controls per case in an attempt to mitigate this issue. It was also difficult to determine if cases represented true clinical infection or colonization and controls could have had other non-MDRO infections. However, our data supports the ongoing need for strong infection control and antibiotic stewardship programs in Indian hospitals. This study could help guide future studies to assess dietary and other risk factors for MDRO colonization specifically in this population or in regions with higher rates of *C. difficile*, by prospectively sampling body sites of interest and comparing against those with no infection or infection with non-MDRO organisms.

In conclusion, this study confirms that antibiotic use is a major driver of MDRO infections and, similar to more developed settings, use of urinary devices increases the risk of MDROs. Our findings suggest that interventions optimizing antibiotic stewardship and reducing device use should be a priority to prevent MDRO infections in this setting.

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Table 1

Frequency of culture type used for diagnoses of cases.

Culture Type	N	%
Urine	17	42.5
Wound	10	25
Blood	6	15
Sputum	3	7.5
Bone	1	2.5
Unlisted	3	7.5

Table 2

Frequency of infection type in cases.

Infection	N	%
<i>Klebsiella pneumoniae spp pneumoniae</i>	13	32.5
<i>Escherichia coli</i>	10	25
<i>Acinetobacter baumannii</i>	5	12.5
<i>Providencia rettgeri</i>	4	10
<i>Psuedomonas aeruginosa</i>	4	10
<i>Psuedomonas luteola</i>	1	2.5
<i>Raoultella onithonolytica</i>	1	2.5
<i>Enterobacter cloacae spp dissolvens</i>	1	2.5
Vancomycin-resistant Enterococci	1	2.5

Table 3

Demographics and medical history.

Variable	Total (n=131)	Controls (n=91)	Cases (n=40)	p-value
Median age (IQR)	57 (42, 65)	56 (40, 63)	58.5 (44.5, 67)	0.213
Male sex (%)	82 (63)	60 (66)	22 (55)	0.234
Median BMI (IQR)	25 (23, 30)	25 (23, 30)	28 (23, 30)	0.617
Median Mid Arm Circumference (IQR)	28 (25, 30)	27.5 (25, 30)	28.5 (31, 25)	0.523
ICU status (%)	12 (9)	6 (7)	6 (15)	0.124
Mean Charlson Comorbidity Index (95% CI)	2.3 (1.9–2.6)	2.1 (1.7–2.5)	2.5 (2.0–3.0)	0.292
Connective Tissue Disorder, n (%)	2 (2)	0 (0)	2 (5)	0.027
Median Length of Stay, days (IQR)	5 (3, 9)	4 (2, 7)	7 (3.5, 12)	<0.001
Hospitalized in past 12 months, n (%)	60 (46)	35 (38)	25 (63)	0.006
Family Member in Hospital, n (%)	19 (15)	10 (11)	9 (23)	0.047
Proton Pump Inhibitor use, n (%)	98 (75)	68 (75)	30 (75)	0.679
Antibiotics in the hospital, n (%)	92 (70)	58 (64)	34 (85)	0.009
Statins, Steroids, H2 Blockers use, n (%)	31 (24)	18 (20)	13 (33)	0.073
Antibiotics in last 12 months, n (%)	43 (33)	25 (27)	18 (45)	0.016
Feeding Tube, n (%)	6 (5)	2 (2)	4 (10)	0.041
Central Venous Line, n (%)	19 (15)	8 (9)	11 (28)	0.003
Urinary Catheter, n (%)	30 (23)	12 (13)	18 (45)	<0.001

BMI: body mass index; MAC: mid-arm circumference; CTD: connective tissue disease; PPI: proton pump inhibitor

Table 4

Mean dietary intake of fiber, fruit, and vegetables, and prevalence of vegetarian status.

Variable	Cases	Controls	P-value
Mean Fiber (g)	2577.2	3257.9	0.599
Mean Fruit (servings)	466.0	527.3	0.897
Mean Vegetable (servings)	1031.4	1285.3	0.573
Vegetarian (n, (%))	16 (40.0)	25 (27.5)	0.160

Table 5

Final adjusted model including predictors with a $p < 0.2$ in the regression model.

Variable	OR	95% CI
Age	2.03	0.99–1.06
Female	3.16	0.77–4.97
Charlson Comorbidity Index	1.23	0.95–1.59
Hospitalized family member	3.16	0.97–10.32
Antibiotic use in last 12 months	3.30	1.22–8.91
Urinary Catheter Use	3.63	1.14–11.58