

Sensitivity Pattern of Gram Negative Bacteria Contaminating Traumatic Wounds: A Retrospective Study in a Tertiary Care Hospital of Kathamandu Valley

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ABSTRACT

Introduction: Gram negative bacteria are commonly involved in wound infections and are significantly associated with morbidity and mortality because of increasing resistance to commonly used antibacterial agents. This study aims to evaluate the pattern of antibacterial sensitivity of the gram negative bacteria from wound swabs of accidental trauma cases.

Methods: This retrospective study was done by collecting all the culture positive reports for wound swabs of accidental wounds from the microbiology laboratory and analyzing the reports showing growth of gram negative bacteria for frequency of sensitivity to commonly used antibacterial agents.

Results: Culture positivity rate was 21.95% with growth of gram negative bacteria in 79.7% of cases. The most commonly isolated organism was *Escherichia coli* being 41.3% among gram negative isolates. Highly resistant bacteria were *E. coli*, *Klebsiella pneumoniae* and *Acinetobacter spp.*

Conclusions: As there is low culture positivity rate for wound swabs obtained before washing the wounds inflicted by accidental trauma, prolonged routine use of prophylactic antibiotic is not justified. Thoroughly washing the wound is very likely to decrease the rate of wound infection further and reduces the need of antibacterial agents. Culture and sensitivity test should guide the treatment when wound is infected because highly resistant gram negative organism may be involved in such cases.

Key words: colistin; culture and sensitivity; gram negative bacteria; multi-drug resistance

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INTRODUCTION

Wound infections continue to represent a major medical problem, both in terms of how they affect the outcome of surgical procedures (surgical site infections), and for their impact on the length of hospital stay and medical costs.¹ Gram negative wound infections are important cause of morbidity and mortality in case of accidental wound infections such as road traffic accidents which are contaminated wounds.² Emerging resistance especially multidrug resistance among aerobic Gram negative bacilli e.g. *Acinetobacter spp.*, *Pseudomonas spp.*, *Klebsiella spp.* is a big challenge and remains as one of the most important factor resulting in poor outcome of patients with wound infection. Gram negative infections may lead to sepsis with relatively high mortality as they are more commonly associated with septic shock and multi-organ failure. High degree of resistance in gram negative bacteria is a global problem and a study done in a tertiary care hospital in Kathmandu too has confirmed this in health care associated infections as well as pyogenic wound infections.^{3,4} Early recognition and appropriate antimicrobial treatment is very essential to prevent such complications and mortality. Though new modalities of treatment e.g. dressing with honey, have been found to be promising in management of wound infections treatment of more deeper infection and prevention of sepsis is still challenging.⁵

This study aims to find sensitivity pattern of gram negative bacteria isolated from accidental wounds in a tertiary care hospital in Lalitpur, Nepal. This may be helpful not only in understanding the evolution of antibacterial resistance in our country but also in developing guidelines for empirical treatment of wound infections based on local susceptibility profiles and developing strategies for containment of antimicrobial resistance that includes cycling the antimicrobial agents to preserve sensitivity.⁶

METHODS

This retrospective study was conducted from the records of culture and sensitivity reports for bacterial isolates in wound swab that arrived at the Microbiology laboratory from the patients visiting the emergency department of a tertiary care

Hospital of Lalitpur, Nepal because of accidental trauma resulting in apparent wound infection. Ethical approval from the Hospital IRC for the study was obtained in February 2020. The culture positive reports with growth of gram negative bacteria from July 2014 to July 2015 were collected and analysed descriptively for frequency of sensitivity and resistance of the gram negative bacteria using Microsoft Excel (Microsoft Office 2016).

Wound swabs were collected, before wound washing, with the help of sterile disposable cotton swabs and immediately inoculated onto blood agar and MacConkey agar media and incubated at 37°C for 24 hours. If there was no growth in 24 hours, the plate was observed at 48 hours for bacterial growth. Identification of bacteria from positive cultures was done with standard microbiological techniques which included Gram staining and biochemical reactions. The antibiotic sensitivity test of all isolates was performed by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar using antibiotic discs of gentamicin (10 µg), amikacin (30 µg), ceftriaxone (30 µg), cefoperazone with sulbactam (75 / 30 µg), imipenem (10 µg), meropenem (10 µg), piperacillin with tazobactam (100/10 µg), colistin sulfate (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg,) chloramphenicol (30 µg) and ampicillin (10 µg) as far as applicable to the isolated gram negative organism. Ceftazidime (30 µg) was used in place of ceftriaxone for *Pseudomonas aeruginosa* (Hi Media Laboratories P. Ltd. Mumbai, India). Interpretations of antibiotic susceptibility results were made according to the guidelines of interpretative zone diameters of CLSI except for colistin.⁷ *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as the control organisms for antibiotic sensitivity.

RESULTS

Culture positivity rate was 21.95% (79 / 360). Rate of infection with gram negative bacteria was 79.7% (63 / 79). The most commonly isolated organism was *Escherichia coli*, which was 41.3% among gram negative isolates. Other gram negative bacteria isolated were *Acinetobacter spp.*, *Klebsiella pneumoniae*, *Enterobacter spp.*, *Pseudomonas aeruginosa*, *Proteus vulgaris*,

Table 1. Sensitivity of gram negative isolates (in percentage)

Antibacterial agents	E.coli (n=26)	Acinetobacter spp. (n=11)	Klebsiella pneumoniae (n=9)	Enterobacter spp. (n=9)	Pseudomonas aeruginosa (n=4)	Others (n=4)
Gentamicin	84.6	63.6	66.7	88.9	100	50
Amikacin	92	63.6	66.7	88.9	100	50
Piperacillin + tazobactam	73	45.5	55.6	66.7	75	75
Ceftriaxone	80.8	27.3	44.5	88.9 *		25
Cefoperazone + sulbactam	80.8	63.6	55.6	55.6	50	50
Ceftazidime	-	-	-	-	50	-
Meropenem	80.8	45.5	77.8	88.9	100	75
Imipenem	61.5	54.6	66.7	55.6	75	50
Ofloxacin	80.8	54.6	66.7	88.9	100	75
Ciprofloxacin	76.9	54.6	66.7	88.9	100	75
Chloramphenicol	-	-	33.3	88.9	*	50
Colistin	96.2	100	100	100	100	100
Ampicillin (Amoxicillin)	7.7	-	*	*	*	*

- = Not tested. * = Intrinsically resistant

Proteus mirabilis, *Enterobacter cloacae*, *Klebsiella oxytoca* (One isolate each for last four organisms included as others in the table). Sensitivity rate of the gram negative isolates for different antibacterial agents is presented in Table 1. Highly resistant *E. coli*, *Klebsiella pneumoniae* and *Acinetobacter* spp., two cases for each, sensitive only to colistin sulfate were isolated during the study period. There was one isolate of *E. coli* resistant to colistin sulfate but sensitive to all other tested agents except ampicillin. Emergence of multidrug-resistant (MDR) *E. coli* has been reported from different parts of the world and it is taken as urgent global health threat due to the lack of effective antimicrobial agents in recent decades.⁸ Resistance to imipenem in *Enterobacter* species is 44.4% which has not been reported by other studies done in Nepal.^{4,9-12}

DISCUSSION

Although study on the postoperative wound infection done in another tertiary care hospital in Kathmandu has shown the rate of Gram positive infections to be higher (57.4%) than Gram negative infections, this study shows the rate of Gram negative infections to be higher.⁴ This can be expected as organism involved in post-traumatic wound infection and surgical wound infections differ. The rate of gram negative infections is shown to be higher in a study done in India on wound infections in road traffic accident patients.¹²

Colistin, an old antibacterial, largely abandoned for systemic use because of systemic toxicity, has been reintroduced in this decade to treat serious multidrug resistant gram negative infections especially those with *Pseudomonas* and *Acinetobacter* species which are resistant to other classes of antibacterial agents.¹² *Klebsiella pneumoniae* sensitive to colistin only (with MIC for imipenem 32 µg/ml) had been isolated from central venous catheter in a case report of 2002 from Greece in a patient with multiple injuries from road traffic accident.¹⁵

Because colistin resistance has already been reported in some parts of the world, it may be expected in this region too though not reported yet.^{4,9-12,15} Combination of colistin with meropenem is shown ineffective in preventing emergence of resistance to colistin in a study. So judicious use of this valuable antibiotic seems to be the way to preserve sensitivity of the highly resistant organisms.¹⁶

Beta lactamases are the hydrolytic enzymes that destroy the functional part of β-lactam antibiotics used for the treatment of most of the Gram negative as well as Gram positive bacterial infections. Alarming increasing resistance in Gram negative isolates towards the extended spectrum penicillins, cephalosporins and carbapenems could be attributed to ESBL, Amp C β-lactamase,

carbapenamase producers and some other relevant underlying mechanisms.¹⁷

Among the orally effective agents, ofloxacin and ciprofloxacin (second generation fluoroquinolones) were tested as most of the Gram negative bacteria are expected to be sensitive to them. The rate of sensitivity to ofloxacin as well as ciprofloxacin was comparable to meropenem for most of the isolated gram negative bacteria and better than imipenem for *E. coli* and *Enterobacter spp.* In addition, *Pseudomonas aeruginosa* isolates which were resistant to ceftazidime (Two out of four isolates) were still sensitive to both of the tested fluoroquinolones. Though the number of isolates is small and better conclusions can be drawn from large scale studies, reserving the fluoroquinolones may be prudent for maintaining the sensitivity of these bacteria towards this valuable group of antibiotics.

CONCLUSIONS

Gram negative bacilli are commonly involved in post-traumatic wound infections and they frequently show multi-drug resistance. Use of

appropriate antibiotics for prophylaxis should be guided by local sensitivity data and treatment of such infections should be guided by wound swab culture and sensitivity report as far as possible. Routine prolonged course of single or multiple antibacterial agents for prophylaxis of wound infection is not justified because the rate of culture positivity is low even with the swabs taken before washing the wounds as shown by the study. Reservation of fluoroquinolones for treatment of serious Gram negative infection may be advisable in light of high sensitivity rate of Gram negative bacteria involved in wound infection. Since the local sensitivity data has prominent role in development of guidelines for treatment of wound infection empirically, similar large scale studies would help in developing such local guidelines minimising unnecessary use of broad spectrum antibacterial agents and decreasing the development of antimicrobial resistance.

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