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IN-VITRO SUSCEPTIBILITY OF LEVONADIFLOXACIN AGAINST STENOTROPHOMONAS MALTOPHILIA.

Medical Microbiology

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ABSTRACT

Stenotrophomonas a versatile organism occurs in soil and natural environments. However, some strains of S.maltophilia are opportunistic pathogens with multidrug resistant profile. Their occurrence in a cancer cohort necessitates looking at their drug susceptibility pattern. A prospective susceptibility testing of 68 non-duplicate isolates of Stenotrophomonas maltophilia to levonadifloxacin was performed and their in vitro susceptibility was compared with other antibiotics as per CLSI guidelines. The 68 isolates of S. maltophilia showed varied susceptibility to trimethoprim/sulfamethoxazole levofloxacin, levonadifloxacin, and minocycline. While 82.36% of isolates showed highest sensitivity to levonadifloxacin 8% showed the least susceptibility. Minocycline exhibited 80.88% susceptibility while susceptibility to trimethoprim/sulfamethoxazole is 76.47%. Levonadifloxacin, a new available molecule (WCK771) can be considered as a potential treatment option based on the susceptibility pattern from this study. However, pharmacokinetics, pharmacodynamics studies and multicenter data in large numbers is warranted.

KEYWORDS

Stenotrophomonas, levonadifloxacin, susceptibility, opportunistic infection

INTRODUCTION:

Stenotrophomonas maltophilia earlier known as Xanthomonas maltophilia is an aerobic gram-negative lactose non-fermenting organism. The genus Stenotrophomonas, belonging to the family of Gammaproteobacteriae, has diverse ecological presence. Its ubiquitous nature and presence in aqueous environments can cause it to colonise on medically invasive devices. Stenotrophomonas maltophilia (S.maltophilia) being the most widely studied species of this genus, is associated with globally emerging infections. The World Health Organisation has recognised infections with these species to be an underestimated Multi Drug Resistant problem, prevalent in the hospital environments. [1][2] Cancer patients often warrant prolonged hospital stay, multiple invasive lines for long term care, neutropenia, mucositis and use of broad-spectrum antibiotics as empirical therapies thereby putting them at higher risk of acquiring infections. S. maltophilia inherently resists widely used antibiotics by different mechanisms such as low membrane permeability, active efflux pumps and secretion of antibiotic-modifying enzymes.[3][4]Carbapenems are amongst the commonly prescribed empirical therapy based on local antibiograms in an immunocompromised cohort. S. maltophilia shows resistance through enzymes like penicillinase (L1) and class A serine cephalosporinase rendering it ineffective to carbapenems.[5][6] Such situations can select such species due to these resistance mechanisms.

As per the CLSI 2024 guidelines'[7]systematic tier reporting, Levofloxacin, Minocycline and Trimethoprim-sulfamethoxazole are the drugs of choice for combating S. maltophilia infections. The cornerstone for treatment of S. maltophilia, historically is with trimethoprim-sulfamethoxazole (TMP-SMX) however, its use as mono therapy is to be avoided as per the CLSI 2024 guidelines. Furthermore, patients in an oncology setup are routinely prescribed low dose of TMP-SMX as a prophylactic antimicrobial cover to prevent infections such as those with Pneumocystis jirovecii. The lingering issue in defining the optimal treatment for S.maltophilia infections is due to low quality of underlying evidence, owing to the lack of randomized clinical trials (RCTs) and inconsistent correlation between in vitro data and clinical outcomes.[8]Also, TMP-SMX has a wide range of side effects namely skin reactions, bone marrow suppression, dyselectrolytemia, hepatotoxicity, nephrotoxicity in patients with pre-existing renal issues, to name a few.

Levofloxacin, a fluoroquinolone antibiotic primarily acts by inhibiting essential enzymes: like DNA gyrase and topoisomerase IV. This causes DNA damage and subsequent cell death secondary to inability of the cell to reproduce. A few new studies observed fluoroquinolones susceptibilities down to 73%[9].

Minocycline, a tetracycline derivative is an important option in the treatment against S.maltophilia. It binds to the 30S ribosomal subunit and impairs protein synthesis. The CLSI 2024 guidelines have lowered the susceptibility breakpoints for minocycline against S. maltophilia from $\leq$ 4 mg/L to $\leq$ 1 mg/L. With this change in breakpoints, susceptibility to minocycline dropped significantly from 77% to 35%. [10] In the wake of this change, minocycline's dependency has been questioned for treating S. maltophilia infections.

The IDSA 2024 guidelines[11] suggest alternative therapies for treating S. maltophilia infections and could be one of the following approaches:

1. The use of any two of the following antimicrobials: cefiderocol, minocycline, TMP-SMX or levofloxacin.
2. Combination of ceftazidime-avibactam and aztreonam.

The L1 metallo-ß-lactamase is constitutively expressed in S.maltophilia to which aztreonam is stable but easily hydrolysed by class A serine L2 enzyme. Avibactam inhibits the L2 enzyme and protects aztreonam. Very few studies have used ceftazidime-avibactam with aztreonam to treat S. maltophilia infections.[12] A successful microbiological eradication was seen when an immunocompromised patient with idiopathic medullary aplasia, complicated with multidrug-resistant S. maltophilia bacteremia was treated for 25 days with aztreonam and ceftazidime-avibactam.[13] The combination of ceftazidime-avibactam with aztreonam is sought after treatment option for treating infections with organisms harbouring metallo-β-lactamases (Class B as per Ambler classification).

However, this combination was not used in our study and robust data pertaining to its efficacy will help establish the viability of this option.

Cefiderocol in a country like India is available only for compassionate use in pan resistant infections. Also, the high cost of the drug raises doubts on its availability in clinical practice. Newer antibiotic approaches, including cefiderocol and aztreonam-avibactam, are promising alternatives for extensively drug-resistant isolates; however, supporting clinical outcomes data is desired. [12] Thus more effective drugs against bugs like S.maltophilia are needed in our armamentarium.

Levonadifloxacin, a benzo-quinolizine subclass of fluoroquinolone antibiotic, a bacterial topoisomerase inhibitor, is used to treat a wide variety of bacterial infections. It inhibits bacterial DNA gyrase and topoisomerase IV, which interrupts the vital DNA replication.

Levonadifloxacin, a new molecule licensed for clinical use in India in 2019 is available in intravenous (WCK 771) and oral formulations

(WCK 2349). It is largely recommended to treat skin and soft tissue infections and bacteraemia. [14] Levonadifloxacin shows enhanced activity in an acidic environment, better intracellular activity and excellent lung pharmacokinetics demonstrating promising potential for treating respiratory isolates.[15,16] The drug is licensed for treatment of adults ≥ 18 years of age. This study was carried out with an aim to determine the in-vitro susceptibility of Levonadifloxacin against S. maltophilia.

MATERIALS & METHODS:

This observational study done in the Department of Microbiology, Tata Memorial Hospital, comprehended data from January 2023 to December 2023. A total of 68 non-duplicate clinical isolates of S.maltophilia from cancer patients recovered from infection sites like blood cultures, respiratory samples and deep seated soft-tissue injuries were considered. Stenotrophomonas maltophilia was identified and its susceptibility to antimicrobial agents was determined by Vitek-2-Compact (BioMe´rieux, Germany).As per the Clinical and Laboratory Standards Institute (CLSI-M10034th Edition)[7]. Pseudomonas aeruginosa ATCC 27853 and Escherichia coli ATCC 25922 were used as control strains meant for the quality check for levonadifloxacin. Antimicrobial susceptibility to levonadifloxacin was done by Kirby Bauer's disc diffusion method using commercially available discs of strength 10µg.

Table 1: The susceptibility breakpoints of the drugs as mentioned in CLSI 2024[7]

Sr. No	Antimicrobial agent	Susceptibility profile			Method of susceptibility testing
		Sensitive	Intermediate	Resistant	
1.	Trimethoprim-sulfamethoxazole	≤40µg/mL	-	≥80 µg/mL	MIC values
2.	Levofloxacin	≤2 µg/mL	4 µg/mL	≥8 µg/mL	MIC values
3.	Minocycline	≤1 µg/mL	2 µg/mL	≥4 µg/mL	MIC values
4.	Levonadifloxacin*	≥17 mm	14-16 mm	≤13mm	Zone disc diameter

* Levonadifloxacin breakpoints extrapolated from Levofloxacin zone disc diameters.

The clinical data derived from the electronic medical records of the hospital was entered in MS office Excel (Microsoft Corp.) and descriptive statistics and correlation matrix is determined for levofloxacin and levonadifloxacin.

RESULTS:

A total of 68 isolates from clinical samples received in the laboratory were confirmed by Vitek2 to be S. maltophilia. The mean age of the patient population was 50.60 years, and patients below 18years of age were excluded from the study as per the drug recommendations for levonadifloxacin. There were 22 females and 46 males in the study. The study focused on understanding the susceptibility pattern of levofloxacin, trimethoprim/sulfamethoxazole, minocycline and levonadifloxacin (table 2) on S. maltophilia. The 68 isolates of S.maltophilia showed comparable sensitivity to the four drugs used in the study. Nevertheless, levonadifloxacin showed 82.36% susceptibility; being the highest. The intermediate sensitive score for levonadifloxacin and levofloxacin respectively is low ranging from 5.88% to 1.47%. On the resistance front, 16 of the isolates (23.53%) show resistance to trimethoprim/sulfamethoxazole combination which was highest and levonadifloxacin exhibits least resistance with 8 isolates (11.76%) out of 68 showing resistance.

Table 2: Antimicrobial Susceptibility Pattern Of Stenotrophomonas Maltophilia Isolated From The Clinical Samples

Antibiotics	Total number of isolates n=68		
	Sensitive	Intermediate sensitive	Resistance
Levonadifloxacin	56 (82.36%)	4 (5.88%)	8(11.76%)
Levofloxacin	53(77.94%)	1(1.47%)	14(20.59%)
Minocycline	55(80.88%)	-	13(19.12%)
Trimethoprim/sulfamethoxazole	52(76.47%)	-	16(23.53%)

A Multiple Antibiotic Resistance (MAR) index is determined for the 68 isolates in the study. A MAR of 0.25 i.e., resistance to 1 antibiotic was 11.76 % (8 isolates), MAR of 0.5 i.e., resistance to two antibiotics and MAR of 0.75 are small namely 5.88% and 1.47% respectively but a MAR of 1.0 shows 11.76%. A MAR greater than 0.2 means that the high risk source of contamination is where antibiotics are frequently used.[17] A MAR index greater than 0.2 exhibited by 30.87% of S. maltophilia is a cause for concern in a tertiary care hospital with immune-compromised patients.

Table 3: Multiple Antibiotic Resistance index of S. maltophilia

MAR index	Number (n=68)
0	47(69.13 %)
0.25	8(11.76%)
0.5	4(5.88%)
0.75	1(1.47%)
1.0	8 (11.76%)

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR= a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility. In the present study b represents the four antibiotics viz., levonadifloxacin, levofloxacin, minocycline and trimethoprim/ sulfamethoxazole.

In the present study the Pearson's correlation matrix depicts the concurrent resistance/susceptibility between levofloxacin and levonadifloxacin (Table 4). The highest coefficient value of 0.992 shows linear relationship between levofloxacin and levonadifloxacin sensitivity confirming their similar mechanism of action and hence relationship. The coefficient for resistance variable also depicts a value of 0.880 and confirms the linear relationship. The p value computed by regression in excel for levonadifloxacin and levofloxacin resistance is 0.020683 which is <0.05 hence statistically significant and p value computed by regression for levonadifloxacin and levofloxacin sensitivity is 7.43415E-05 which is < 0.05.

Table 4: Correlation matrix of Levofloxacin and Levonadifloxacin for S.maltophilia

	LVX S	LVX I	LVX R	LND S	LND I	LND R
LVX S	1					
LVX I	-0.485094079	1				
LVX R	-0.320444292	-0.57905	1			
LND S	0.9929517 *	-0.5336	-0.22865	1		
LND I	-0.136436435	0.60025	-0.46078	-0.20754	1	
LND R	-0.514199723	-0.5	0.88016	-0.46214	-0.42875	1

A correlation matrix is a table that displays correlation coefficients for different variables. This matrix is created by computing correlation coefficients between clinical factors of interest. The coefficients measure the strength and direction of linear relationship between the variables. The values range from -1 to +1. LVX S: levofloxacin sensitive, LVX I: levofloxacin intermediate, LVX R: levofloxacin resistant, LND S: levonadifloxacin sensitive, LND I: levonadifloxacin intermediate LND R: levonadifloxacin resistant.

DISCUSSION:

The incidence of hospital-acquired S.maltophilia infections are on the rise, though the organism is not a highly virulent pathogen.[1] Hence to keep pace with infections and treat effectively, clinicians have an urgent need to look for new therapies. Nonetheless, absence of clinical outcome data, lack of reproducibility of antimicrobial susceptibility testing and difficulties in laboratory identification hinder treatment recommendations. TMP-SMX is the mainstay of treatment but alternative regimes and combined therapy are necessary to address antibiotic resistance mechanisms.[5] In a study conducted by Mohammad Asraf Ali Namaji et al, 85.7% S. maltophilia isolates show susceptibility to TMP-SMX and minocycline.[18] A study by Hafiz T.A. et al report 95.9% susceptibility to TMP-SMX while levofloxacin report a susceptibility of 68.9%.[19] In the current study, 77.94% isolates of S.maltophilia are susceptible to levofloxacin, followed by 76.47% to TMP-SMX and 80.88% to minocycline. The investigational drug levonadifloxacin showed the highest susceptibility at 82.36%. The structural modifications in levonadifloxacin as compared to levofloxacin, contribute to its improved ability to overcome resistance mechanisms. The presence of efflux pumps and altered target sites diminish the effectiveness of traditional fluoroquinolones like levofloxacin.[20] The most thoroughly explored alternative agent to TMP-SMX is primarily levofloxacin, a fluoroquinolone that offers good susceptibility rate, efficacy and safety.[12]

Limitations: This study is restricted to isolates obtained from cancer patients and is a single centre study with susceptibility breakpoints of levonadifloxacin extrapolated from levofloxacin breakpoints. Comparative analysis with newer combination options should be carried out to establish efficacy. Robust in-vitro studies targeting the specific pharmacokinetic and pharmacodynamic studies will help establish accurate breakpoints. Furthermore, a molecular workup to understand the exact mechanism of resistance and resistance markers will be required for validation of the results.

CONCLUSION:

Levonadifloxacin is shown to have a broad spectrum of antibiotic cover predominantly including gram positive as well as a few gram-negative organisms. This will help in a one-stop solution for polymicrobial infection treatment. However, RCT pertaining to in-vivo studies with clinical correlation are needed to establish a standardised dosing regimen. While a multi-centre study including a greater number of clinical isolates is needed to prove definitive activity of levonadifloxacin against gram-negative organisms, this could be considered as a potential treatment modality.

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