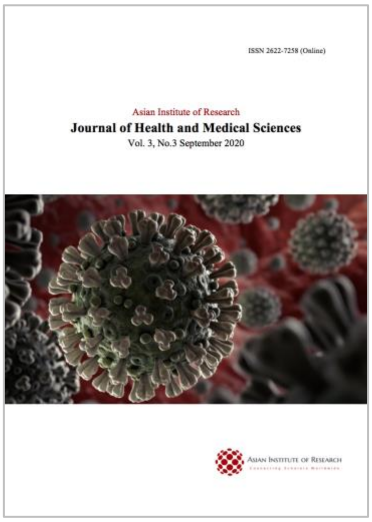

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Antimicrobial Susceptibility Pattern of Urine Culture Isolates in a Tertiary Care Hospital of Karachi, Pakistan

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Abstract
Background: Urinary Tract Infections (UTIs) remain one of the leading causes of infection worldwide, andare a major health concern in developing countries, and lead patients to seek medical care. Recent reports have shown increasing resistance to commonly-used antibiotics. We aimed to study the isolates and their antibiotic resistance pattern in outpatients. Objective: The study was done in Karachi on outdoor patient urine samples, of patients with clinically suspected UTIs, in order to assess the antimicrobial susceptibility pattern of different organisms, which would help in deciding empirical antibiotic treatment and improving patient outcome. Materials and Methodology: 400 urine samples of patients clinically suspected to have UTI were collected directly from the bacteriology lab, out of which 77 were culture positive. The positive samples included 64 females and 13 males. Results: The most commonly isolated organism was *Escherichia coli (E.coli)*, followed by *Klebsiella species*. *Escherichia coli was* most sensitive to meropenem and nitrofurantoin (96.6%) whereas *Klebsiella* species were sensitive to several antibiotics excluding cotrimoxazole and the fluoroquinolones. Conclusion: *E.coli species* is the most common organism causing UTIs. Antimicrobial resistance is emerging against some antibiotics, and the current susceptibility patterns may be used locally for optimum therapeutic outcomes and for preventing antibiotic misuse.

Keywords: Antimicrobial Susceptibility, Urine Culture Isolates, Tertiary Care, Hospital

1. Introduction

Urinary tract infection (UTI) describes microbial colonization and infection of structures of the urinary tract. UTI is categorized by infection site as pyelonephritis (kidney), cystitis (urinary bladder), and urethritis (urethra), and can also be classified as uncomplicated or complicated [Ejrnæs K, (2011)]. Urinary tract infections are currently placed among the most widespread infectious diseases worldwide, with chronic and recurrent infections being troublesome [Matthew GB, Matthew AM, (2010)]. Clinical studies suggest that the overall prevalence of UTI is higher in women; less complicated UTIs in healthy women have an incidence of 50/1000/year. UTI varies with age and gender, boys between the ages of 1-5 year suffers UTI more frequently and need to be evaluated efficiently [Gupta P, Mandal J, Krishnamurthy S, Barathi D, Pandit N (2015)].

Uropathogenic *Escherichia coli* (UPEC) are the primary etiologic agents, and the most common cause of urinary tract infections (UTIs) worldwide[R N Das, T S Chandrashekhar, H S Joshi, M Gurung, N Shrestha, P G Shivananda (2006); Ihsan A, Zara R, Safia A, Sajid M, Javid ID, (2015)]. *E.coli*pathotypes reside harmlessly in the human intestinal microenvironment but, upon access to sites outside of the intestine, become a major cause of human morbidity and mortality as a consequence of invasive UTI (pyelonephritis, bacteremia, or septicaemia) [Alteri CJ, Mobley HL (2015)]. The virulence factors of *E. coli* are multiple and unusually complex affecting pathogenicity in combination with one another. [Hegde A, Bhat GK, Mallya S, (2008)].*Escherichia coli* expresses multi-drug resistance. In the treatment of uncomplicated cystitis, the preferable antibiotics for empiric treatment include nitrofurantoin, trimethoprim/sulphamethoxazole, or ciprofloxacin. The alternate choices include cefuroxime and cefixime. For complicated and upper UTI cases in hospitalized patients, the antibiotics used are piperacillin/tazobactam and carbapenems[Jharna M, Srinivas AN, Buddhapriya D, Subhash CP, (2012)].

Antibiotic resistance among bacteria causing common infections is increasing in all regions of the world [Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century (2014)]. Infections are gradually becoming hard to treat, and may lead to therapeutic dead-ends [Moroh JLA, Fleury Y, Tia H, et al, (2014)]. The emergence of resistance to antibiotics illustrates the importance of using evidence-based strategies for treatment [Nickel JC, (2005)]. In UTI cases, antibiotic treatment is often started empirically before the results of urine culture and susceptibility testing are available. Appropriate antibiotic use in patients with UTI seems to reduce length of hospital stay and therefore favors patient outcomes and healthcare costs[Spoorenberg V, Hulscher ME, Akkermans RP, Prins JM, Geerlings SE, (2014)]. Hence, it becomes important to regularly monitor the resistance or susceptibility patterns of uropathogens, so that the guidelines for empirical antibiotic therapy can be improved to include antibiotics with low resistance, aiding clinicians in proper management of UTIs with minimal therapeutic failures[Sharma N, Gupta A, Walia G, Bakhshi R, (2016)]. The antibiotic resistance patterns have shown large inter-regional differentiation. The appropriate choice of antibiotic needs to be tailored based on the local susceptibility pattern[Prasada S, Bhat A, Bhat S, Shenoy Mulki S, Tulasidas S, (2019)]. Factors such as the type of UTI (complicated or uncomplicated), gender, age, and previous history of antibiotic therapy of each UTI patient should also be considered to find out the correct global data on susceptibility and for further appropriate treatments attempts [Alos JI (2005)]. Data provided by regional microbiology laboratories on the susceptibility patterns helps to choose the empirical choice of antimicrobials to treat UTI [McNulty CAM, Richards J, Livermore DM (2006), Car J (2006)]. Generally, the antimicrobial treatment is initiated before the laboratories results which may lead to the frequent misuse of antibiotics [Tambekar DH, Dhanorkar DV, Gulhane SR, Khandelwal VK, Dudhane MN, et al. (2006)].

All over the world, resistance against beta-lactam antibiotics is increasing due to Extended Spectrum Beta Lactamases (ESBLs) and Amp-c beta-lactamase production. Carbapenemases are plasmid-encoded and has reduced the activity of all penicillins, monobactams, cephalosporins and carbapenems[Arpin C, Dubois V, Coulange L, Andre C, Fischer I ,Noury P et al. (2003)]. Beta lactamases cause resistance to beta-lactam agents and are produced by different aerobic gram-negative bacteria (AGNB) [Aggarwal R, Chaudhary U, Sikka R (2004)]. ESBLs were discovered in 1980. The main reservoirs for these resistant organisms are hospital patients[Wiener J, Quinn J, Bradford P, Goering R, Nathan C, Bush K, (1999)].

Keeping in view the aforementioned considerations, this study was conducted with the aim of reviewing current antimicrobial sensitivity and resistance pattern in males and females of different age groups, therefore contributing to the prevention of therapeutic failures and antibiotic misuse in patients with UTI.

2. Materials and Methods:

2.1 Study area and population:

This cross-sectional study was carried out at the Ziauddin Hospital in Karachi, Pakistan. This hospital caters to the needs of patients from nearby urban and rural localities as well as from other parts of the province. The study included outdoor patients' urine samples collected over a period of one year from Jul 1, 2019 –Jun 30, 2020. The patients had clinical evidence of urinary tract infection as suggested by their physician. Culture and susceptibility reports were obtained directly from the micro-biology lab.

2.2 Sample size:

The urine samples of 400 patients comprising 315 females and 85 males were included in the study. Out of the 400 samples processed, 77 (19%) depicted bacterial growth, 28(7%) showed mixed flora, and the rest of the 374 (74%) samples were found to be sterile.

2.3 Sample collection and processing:

Patients were instructed to collect midstream urine sample in a bottle and give it to the laboratory for further processing. In the lab, the organisms were isolated and the colony count of each organism was measured. Culture results were interpreted as being significant and insignificant, according to the standard criteria. A growth of $>10^5$ colony forming units/mL was considered as significant bacteriuria.

2.4 Anti-microbial susceptibility testing:

17 antibiotics were tested as part of the study including, amikacin, amoxicillin-clavulanic acid, ampicillin, aztreonam, cefoperazone/sulbactam, cefixime, cefotaxime, ceftriaxone, colistin, co- trimoxazole, gentamicin, imipenem, meropenem, nitrofurantoin, ofloxacin/ciprofloxacin, polymixin and tazobactam/piperacillin. All the microscopic examinations involving identification of bacterial strains was performed by authorized laboratory technicians. Appropriate quality control strains were used to validate the results of the antimicrobial discs. The data was entered into Microsoft Excel and analyzed. The results were expressed as proportions and mean susceptibility was calculated for the antibiotics for each organism.

3. Results:

Table 1. Age and gender wise distribution of urine sample

Age (Years)	Gender		Total
	Female	Male	
0 – 20	7	1	8 (10.4%)
21 – 40	24	2	26 (33.8%)
41 – 60	17	1	18 (23.4%)
61 – 80	13	7	20 (26.0%)
81 – 100	3	2	5 (6.5%)
Total	64 (83.1%)	13 (16.9%)	77

3.1 Prevalence rate and frequency distribution of UTI among males and females in different age groups:

The total prevalence of UTI was found to be 83.1% in females and 16.9% in males as seen in Table 1, hence indicating a higher prevalence in femalepatients. The highest susceptible age group of UTI patients irrespective of gender was found to be 21-40 years (33.8%) followed by 61-80 years (26.0%), and then between 41-60 years (23.4%). The lowest prevalence of UTI was found in age groups of 0-20 years (10.4%), followed by 81-100 years (6.4%), irrespective of gender.

The prevalence of UTI differed in males and females according to age groups. The highest prevalence in females was found in the age group of 21-40 years, whereas elderly males in the age bracket of 61-80 years were mostly affected.

On the contrary, elderly females (81-100 years) were least affected by UTI. The least susceptible groups in males were those between 0-20 years and 41-60 years.

Table 2. Gender wise bacterial isolates

Bacterial Isolates	**Gender**				**Total**
	Female		**Male**		
	n	**%**	**N**	**%**	
Enterobacter Species	1	33.3%	2	66.7%	3
Enterococcus Species	3	100.0%	0	0.0%	3
Escherichia Coli	38	86.4%	6	13.6%	44
Klebsiella Species	12	80.0%	3	20.0%	15
Morganella Morganii	1	100.0%	0	0.0%	1
Pseudomonas aeruginosa	5	83.3%	1	16.7%	6
Serratia Species	1	100.0%	0	0.0%	1
Staphylococcal species	1	100.0%	0	0.0%	1
Streptococcus Group D	1	50.0%	1	50.0%	2
Streptococcus Species	3	100.0%	0	0.0%	3

3.2 Distribution frequency of isolated bacterial uropathogens:

As seen in table 2, among all the isolated bacterial uropathogens from UTI patients, *Escherichia coli* was found as the dominant bacteria with the highest prevalence, irrespective of gender. The second most prevalent isolate was *Klebsiella sp.* followed by *Pseudomonas*. *Enterococci sp, Enterobacter* and S*treptococcus sp.* had the same prevalence. The organisms with the lowest prevalence were found to be *Serratia sp.* along with *Morganella Morganii* and *Staphylococcal sp,* irrespective of gender.

3.3 Gender-wise distribution of uropathogens

The prevalence rate for the occurrence of different uropathogens among males and females had some variations. *Enterococcus species,* for example, was only seen in females (100%), whereas no isolates were found in males. *Morganella morganii, Serratia species, Staphylococcal* and S*treptococcal species* followed the same pattern.

On the other hand, some organisms had a higher prevalence in females as compared to males. These include *E. coli*with a prevalence of 86.4% in females and 13.6% in males, followed by *Pseudomonas aeruginosa*, the prevalence of which was 83.3% in females and 16.7% in males. *Klebsiella species* was also more prevalent in females (80%) and only 20% of the isolates were found in males.

Enterobacter species was the only organism which had higher prevalence in males (66.7%) than in females (33.3%). *Streptococcus groupD*was equally prevalent in males and females.

Table 3. Isolates from all the urine samples and their susceptibility pattern to antibiotics

ANTIBIOTICS	BACTERIAL ISOLATES												
	Citrobacter species	Enterobacter Species	Enterococcus Species	Escherichia Coli	Klebseiella Species	Morganella Morganii	Providencia Species	Pseudomonas aeruginosa	Salmonella species	Serratia Species	Staphylococcal species	Streptococcus Group D	Streptococcus Species
AMIKACIN	100.0%	80.0%	100.0%	87.9%	87.5%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
AMOX-CLAV	100.0%	80.0%	100.0%	72.4%	81.3%	0.0%	100.0%	100.0%	0.0%	100.0%	0.0%	100.0%	100.0%
AMPICILLIN	100.0%	80.0%	80.0%	25.9%	87.5%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%	66.7%	100.0%
AZTREONAM	100.0%	40.0%	100.0%	37.9%	62.5%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
CEF-SUL	100.0%	100.0%	100.0%	87.9%	81.3%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
CEFIXIME	100.0%	40.0%	100.0%	34.5%	62.5%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
CEFOTAXIME	100.0%	40.0%	100.0%	37.9%	62.5%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
CEFTRIAXONE	100.0%	40.0%	100.0%	37.9%	62.5%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
COLISTIN	100.0%	80.0%	100.0%	84.5%	100.0%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
CO-TRIMOXAZOLE	0.0%	80.0%	100.0%	50.0%	62.5%	100.0%	0.0%	100.0%	0.0%	100.0%	0.0%	100.0%	100.0%
GENTAMICIN	100.0%	80.0%	100.0%	81.0%	81.3%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
IMIPENEM	100.0%	100.0%	100.0%	94.8%	93.8%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
MEROPENEM	100.0%	100.0%	100.0%	96.6%	93.8%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
NITROFURANTOIN	100.0%	80.0%	80.0%	96.6%	68.8%	100.0%	100.0%	100.0%	0.0%	0.0%	100.0%	100.0%	100.0%
OFLOX/CIPRO	100.0%	60.0%	100.0%	46.6%	68.8%	0.0%	0.0%	66.7%	0.0%	100.0%	0.0%	100.0%	100.0%
POLYMIXIN	100.0%	80.0%	100.0%	84.5%	100.0%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%
TAZO/PIPERA	100.0%	100.0%	100.0%	87.9%	81.3%	100.0%	100.0%	100.0%	0.0%	100.0%	100.0%	100.0%	100.0%

Table 4. Isolates from all the urine samples and their resistance pattern to antibiotics.

ANTIBIOTICS	BACTERIAL ISOLATES												
	Citrobacter species	Enterobacter Species	Enterococcus Species	Escherichia Coli	Klebseiella Species	Morganella Morganii	Providencia Species	Pseudomonas aeruginosa	Salmonella species	Serratia Species	Staphylococcal species	Streptococcus Group D	Streptococcus Species
AMIKACIN	0.0%	20.0%	0.0%	12.1%	12.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
AMOX-CLAV	0.0%	20.0%	0.0%	27.6%	18.8%	100.0%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
AMPICILLIN	0.0%	20.0%	20.0%	74.1%	12.5%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	33.3%	0.0%
AZTREONAM	0.0%	60.0%	0.0%	62.1%	37.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CEF-SUL	0.0%	0.0%	0.0%	12.1%	18.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CEFIXIME	0.0%	60.0%	0.0%	65.5%	37.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CEFOTAXIME	0.0%	60.0%	0.0%	62.1%	37.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CEFTRIAXONE	0.0%	60.0%	0.0%	62.1%	37.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
COLISTIN	0.0%	20.0%	0.0%	15.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
CO-TRIMOXAZOLE	100.0%	20.0%	0.0%	50.0%	37.5%	0.0%	100.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%
GENTAMICIN	0.0%	20.0%	0.0%	19.0%	18.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
IMIPENEM	0.0%	0.0%	0.0%	5.2%	6.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
MEROPENEM	0.0%	0.0%	0.0%	3.4%	6.3%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
NITROFURANTOIN	0.0%	20.0%	20.0%	3.4%	31.3%	0.0%	0.0%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%
OFLOX/CIPRO	0.0%	40.0%	0.0%	53.4%	31.3%	100.0%	100.0%	33.3%	100.0%	0.0%	100.0%	0.0%	0.0%
POLYMIXIN	0.0%	20.0%	0.0%	15.5%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
TAZO/PIPERA	0.0%	0.0%	0.0%	12.1%	18.8%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Tables 3 and 4 show the organisms' susceptibility and resistance pattern to the 17 antibiotics that were part of the study.

E.coli was most susceptible to meropenem and nitrofurantoin (96.6%), followed by imipenem (94.8%), cefoperazone/sulbactam, amikacin and tazobactam/piperacillin(87.9%). It was least sensitive to ampicillin (25.9%).

100% of*Klebsiella sp*were sensitive to colistin and polymixin, followed by imipenem and meropenem (93.8%), followed by ampicillin (87%) and amoxicillin-clavulanic acid (83%). They was least sensitive to aztreonam, cefixime, cefotaxime, ceftriaxone and co-trimoxazole (62.5%).

Pseudomonas was among those organisms which were 100% sensitive to most of the antibiotics. It was only resistant to one antibiotic, ofloxacin/ciprofloxacin (33.3%). *Streptococcus species* was the only organism that was 100% sensitive to all the antibiotics. *Streptococcus group D* followed a similar pattern except that it was somewhat resistant to ampicillin – 33% of the isolates.

The other organisms in this category include *Staphylococcal species* were 100% resistant to some antibiotics which included amoxicillin-clavulanic acid, co-trimoxazole and ofloxacin/ciprofloxacin.

Salmonella was sensitive to all antibiotics except ofloxacin/ciprofloxacin to which it was 100% resistant. Similarly, *Serratiasp.*was resistant to only nitrofurantoin. Other organisms which were 100% sensitive to most of the antibiotics include *Providencia sp*,*Morganella sp*, *Enterococcus* and C*itrobacter sp.*.

4. Discussion:

The age and gender distribution of the patients diagnosed with UTI followed the natural epidemiological pattern of UTI, with young females being the most affected group, which is related to the difference between the male and female genitourinary systemsin anatomy and microflora. This study highlights the current scenario of UTI and the anti-microbial susceptibility pattern in the urban and rural settings of other cities in the developing world. In females of all age categories, *E. coli* is the most frequently isolated uropathogen which correlates with other studies [Nys S, van Merode T, Bartelds AIM, Stobberingh EE (2006); Hazarika J, Baruah K, (2018); Nys S, (2005)]. It is followed by*Klebsiella sp.*which is also the second most commonly isolated organism in various studies[George CE, Norman G, Ramana GV, Mukherjee D, Rao T (2015); Prakash D, Saxena RS (2013); Somashekara SC, Deepalaxmi S, Jagannath N, Ramesh B, Laveesh MR, Govindadas D, et al (2014)].

Increasing antimicrobial resistance has been documented in this study as well as globally [Claudia V, Francesca L, Maria PB, Gianfranco D, Pietro EV, et al. (2014); Kashef N, Djavid GE, Shahbazi S (2010); Karlowsky JA, Jones ME, Thornsberry C, Critchley I, Kelly LJ, et al. (2001); Rajalakshmi V, Amsaveni V (2011); Sharifian M, Karimi A, Tabatabaei SR, Anvaripour N (2006); Haghi-Ashteiani M, Sadeghifard N, Abedini M, Soroush S, Taheri-Kalani M, et al. (2007); Rashed Marandi FRM, Saremi M (2008)]. The tendency to self-medicate, noncompliance with treatment, financial constraints and lack of education on the part of patients; the sale of antibiotic drugs without proper prescription and failure to educate patients on the part of pharmacists; negligible surveillance of susceptibility patterns, poor regulatory controls over antibiotics and a lack of will to make a change on part of health care system, and administering antibiotics before obtaining samples for culture, failure to educate patients and poor prescribing practices on part of physicians are among many factors that lead to injudicious and inappropriate use of antibiotics, hence causing the rapid development of resistance [World Health Organization. The World Health Report 1996; World Health Organisation. National Action Plan on Antimicrobial Resistance (NAP-AMR) 2017-2021. New Delhi, India: World Health Organisation Country Office for India; 2017; Laxminarayan R, Chaudhury RR (2016). Porter G, Grills N (2016).].

The above study shows that *E.coli* is most susceptible to meropenem and nitrofurantoin, but it also showed a high sensitivity to amoxicillin-clavulanic acid (72.4%) and hence this maybe the preferred oral drug of choice in *E.coli* positive patients. On the contrary we see that *E.coli* is most resistant to ampicillin (74.1%), and 53% of the isolates were resistant to ofloxacin/ciprofloxacin, therefore these may be excluded in the empiric treatment of *E.coli* positive patients.

For *Klebsiella sp*, which is the second most common uropathogen, a high susceptibility to commonly used antibiotics (amoxicillin and amoxicillin-clavulanic acid) was noted, however, 33% of the isolates were resistant to ofloxacin/ciprofloxacin, and several of the studies quoted above have shown an increased resistance to fluoroquinolones. The resistance of 33% of *Pseudomonas sp* and 40% of *Enterobacter sp* to fluoroquinolones also depicts the widespread use of these antibiotics, even in the absence of a prescription. The 100% resistance of *Citrobacter sp* to cotrimoxazole may be due to the widespread use of this drug in the community.

Resistance to antibiotics is a leading cause of therapeutic failures all over the world. This study was therefore aimed at studying the antimicrobial resistance trends and aiding clinicians in deciding the appropriate empirical treatment, hence improving patient outcome. This may also help in preventing the misuse of antibiotics in UTI patients. A unified antibiotic protocol is necessary to restrict the use of antibiotics injudiciously in order to prevent resistance and reduce the complications of UTI arising from the use of resistant drugs. Since thos was a cross-sectional study, further regular monitoring and a continuous review of antibiograms is also necessary to track changes in etiological agents and antimicrobial patterns to help in empirical treatment.

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