Surveillance of Urine Cultures and Evaluation Gram Negative Uropathogens; Five Year Data from Erbil

Mohammed Sadeq SALEH[1], Hevi Seerwan Ghafour[2], Tayfur Demiray[3], Mustafa Altindis[3]

1 Pirmam General Hospital, Department of Diagnostic Microbiology, Erbil, Kurdistan Region, Iraq

2 Department of Biomedical Sciences, Cihan University-Erbil, Kurdistan Region, Iraq

3 Department of Clinical Microbiology, School of Medicine, Sakarya University, Sakarya, TURKEY

Abstract - Urinary tract infections (UTIs) are most common infectious disease and a public health problem that imposes a large economic burden. The aim of this study is to gather surveillance data of urine cultures and determine the prevalence of uropathogens in urine samples of patients referred to outpatient clinics in Erbil region and to evaluate the antimicrobial susceptibility of the gram negative uropathogens. All urine cultures result of patients referred to Erbil hospitals in the last 5 years (2015-2020) are retrospectively examined in this study. Microorganisms are identified by standard bacterial methods and their susceptibilities are assessed by VITEK 2 automated system. The results of urine culture of 3380 suspected UTI cases are examined and out of 3097 positive cultures observed, a total of 1961 (63.3%) isolates are gram-negative and 1136 (36.7%) are gram-positive pathogens. The most common urinary pathogen determined in this study is Escherichia coli. The highest resistances of gram-negative urinary pathogens are against the ampicillin, trimethoprim/sulfamethoxazole and ceftriaxone. It is thought that the data obtained from this study will be useful in the planning of empirical treatment of urinary tract infections and in the development of rational antibiotic use policies.

Keywords: Urine Culture; Urinary Tract Infections; Antimicrobial Resistance

INTRODUCTION

Urinary tract infections (UTIs) with a significant burden of economic are considered as common human's diseases of infectious and are a general health issue. UTIs, in the United States, are the most common urinary tract disease and are responsible for annual physician visits of more than 7 million and 15% of all antibiotics of community prescribed. Many European countries have similar incidence rates as well. (Köves, (2017)) UTI-related health care costs are more than 3 billion $ per year globally. (Anderson, (2010)) A systematic review by Beyer et al., reports the prevalence of UTIs in eight different countries to be between 17% and 82%. (Beyer, (2019)) Gram-negative bacteria are the most common cause of UTI. The primary pathogens which lead to pyelonephritis and inflammation of uncomplicated bladder are E. coli, and other Enterobacteriaceae strains including K. pneumoniae and P. mirabilis, and gram-positive pathogens including S. saprophyticus and E. faecalis. (Flores-Mireles AL, (2015)) (Ganguly N, (2011)) In women infections of urinary tract are frequent. A reason for this variation between the genders is due to anatomical differences such as a urethra of shorter and the fair urethra proximity to anus. Many other elements are involved, such as intercourse practices of sexual and utilizing spermicides that alter the natural flora of the vagina. (Scholes, (2000)) (T, (2017))

Several medications including fluoroquinolones, fosfomycine, trimethoprim-sulfamethoxazole, nitrofurantoin and beta-lactams are recommended in international

recommendations for the treatment of pyelonephritis and tract infections of uncomplicated urinary tract infections. (Köves, (2017)) (Ganguly N, (2011)) Nevertheless, there is a worrying amount of resistance of antimicrobial in urinary pathogens because of the widespread and indiscriminate applying antibiotics. Broad-spectrum beta-lactamase-producing bacteria show resistance to several antibiotics regardless of carbapenem and are constantly enhancing within people. (Köves, (2017)) (Oteo, (2010).) Evaluation of pathogens and their sensitivity to different antibiotics has a high effect on the empirical treatment of patients with UTI, and if the appropriate antibiotic is selected by the physician, further costs and complications will be avoided. Health policies and rational antibiotic use protocols will be both more reliable and more applicable when created based on surveillance data. The aim of this study is to provide surveillance data for Erbil region and is to evaluate urine culture results according sex and age groups as well as to reveal contamination rates. Also, the prevalence of gram-negative uropathogens and their antimicrobial susceptibilities in urine samples of patients referred to outpatient clinics in Erbil region.

2. Materials and Methods

This cross-sectional-analytical retrospective study was performed to evaluate the results of urine culture of outpatients of Rzgary Teaching Hospital, Hawler Teaching Hospital, General Health Laboratory, and CMC Private Hospital which are all serving Erbil region during the years from 2015 to 2020. A total of 3380 samples were examined. Standard bacterial methods and biochemical tests together with VITEK 2 automated identification system were used to identify uropathogens. VITEK 2 automated system was also used to evaluate antimicrobial susceptibilities of the causative bacteria according to CLSI criteria. The inclusion criteria were the age over 18 years with positive urine culture of outpatients and the exclusion criteria was the age less than 18 years and hospitalized patients.

The results were evaluated based on the sex and age groups of 18-48 years, 46-60 years and more than 60 years. The antibiotic susceptibility panels including ampicillin, cefepime, aztreonam, cefixime, ceftriaxone, ertapenem, amoxicillin/clavulanate, piperacillin/tazobactam, amikacin, cefotaxime, imipenem, meropenem, ceftazidime, nitrofurantoin, levofloxacin, trimethoprim/sulfamethoxazole, ciprofloxacin and gentamicin were tested for the Gram-negative uropathogens.

As a limitation, since this study is retrospective, it was not possible to gather information such as previous or ongoing use of antibiotics which are not routinely questioned or recorded from outpatients.

Statistical Analyses

The data were analyzed using SPSS version 26.0 software (IBM Corp). The results of descriptive analysis were reported by tables and graphs. Chi-square test was used to examine the relationship between variables and a p value less then 0.005 is considered as significant.

3. Results

Total number of 3380 urine cultures were evaluated retrospectively. Out 3380 cultures, 3097 (91.6%) were reported as positive. The contamination was determined in 95 (2.8%) of the cultures (Table 1). When evaluated according to age groups, there was not any significant difference in terms of “no growth”, contamination or positive cultures ($p > 0.005$).

Table 1. Urine culture results according to age groups.

	18 to 45 years	46 to 60 years	>60 years	Total	
No growth	79 (5.3%)	56 (5.8%)	53 (5.6%)	188 (5.6%)	$p > 0.005$
Contamination	41 (2.8%)	22 (2.4%)	32 (3.4%)	95 (2.8%)	
Positive	1369 (91.9%)	877 (91.8%)	851 (91%)	3097 (91.6%)	
Total	1489 (100%)	955 (100%)	936 (100%)	3380 (100%)	

When all urine cultures were evaluated according to gender, 69.1% (n= 1045) of the urine cultures were requested from female outpatients where 30.9% (n= 2335) were from

males. For the positive urine cultures, the ratios were similar and causative bacteria were more isolated from female patients. Moreover, significant difference was determined when positive urine cultures were evaluated according to age groups and sex. Urine culture positivity was higher in 18 to 45 years' female age group compared to the male age group and in total (Table 2).

Table 2. Positive urine culture results according to age and sex groups.

	18 to 45 years	46 to 60 years	>60 years	Total	
Male	104 (3.4 %)	326 (10.5 %)	429 (13.9 %)	859 (27.7 %)	$p < 0.005$*
Female	11265 (40.8 %)	551 (17.8 %)	422 (13.6 %)	2238 (72.3 %)	
Total	1369 (44.2 %)	877 (28.3 %)	851 (27.5 %)	3097 (100%)	

*For the 18 to 45 years' group and when all age groups in total evaluated.

Among the positive urine cultures 1961 (63.3 %) of the isolates were gram-negative and 1136 (36.7%) were gram-positive pathogens. No significant difference was detected but gram-negative dominance was clearly observed between age groups and in total (Table 3).

Table 3. Distribution of gram-negative and gram-positive pathogens according to age groups.

	18 to 45 years	46 to 60 years	>60 years	Total	
Gram-negative	867 (28%)	553 (17.9%)	541 (17.5%)	1961 (63.3%)	$p > 0.005$
Gram-positive	502 (16.2%)	324 (10.5%)	310 (10%)	1136 (36.7%)	
Total	1369 (44.2%)	877 (28.3%)	851 (27.5%)	3097 (100%)	

The most common gram-negative pathogen identified was E. coli, which accounted for 70% of gram-negative isolates. K. pneumoniae (17%), Proteus mirabilis (3.9%) and P. aeruginosa (3.4%) were the following most common pathogens, respectively (Table 4). Among gram-positive pathogens, coagulase negative staphylococcus spp. followed by S. aureus were most commonly isolated microorganisms.

Table 4. Distribution of gram-negative uropathogens

Group	Bacteria	n	%
Fermentative (n=1868)	*E. coli*	1373	70,0
	K. pneumoniae	334	17,0
	P. mirabilis	77	3,9
	E. cloacae complex	27	1,4
	E. aerogenes	25	1,3
	K. oxytoca	12	0,6
	M. morganii	9	0,5
	S. marcescens	5	0,3
	Salmonella group	3	0,2
	Shigella group	3	0,2
Non-fermentative (n=93)	*P. aeruginosa*	67	3,4
	S. paucimobilis	4	0,2
	A. baumannii cplx	13	0,7
	A. haemolyticus	4	0,2
	A. lwoffii	5	0,3
Total		1961	100,0

Of Escherichia coli isolates, 67.7% are resistant to Ampicillin, 52% to TMP / SMX, 32.7% to ceftriaxone and 31.4% to Cefixime. The lowest antibiotic resistance was reported to Escherichia coli for the antibiotics Amikacin (0.2%), Meropenem (0.3%), Imipenem (0.4%) and Ertapenem (0.4%). These four antibiotics also showed the highest sensitivity in other isolates. The pattern of resistance of antibiotics to K. pneumoniae was largely similar to that of E. coli. In Enterobacter cloacae complex isolate, the highest resistance was related to Amoxicillin / Clavulanate. Resistance to Ertapenem and Levofloxacin was reported in only three types of gram-negative urinary pathogens and the rest of the strains were quite sensitive. Only S. marcescens and Salmonella strains showed no resistance to Ciprofloxacin, and resistance to this antibiotic was observed in other strains (Table 5).

Of the E. coli isolates, 38.6% were broad-spectrum beta-lactamase-producing strains. However, the strains with the highest ESBL production were Shigella group (66.7%; 2 out of 3), K. oxytoca (41.7%; 5 out of 12) and K. pneumonia. (40.1%; 134 out of 334 cases). Overall, 35.5% (696 cases) of gram-negative isolates produced ESBL.

Table 5: Antimicrobial resistance of gram-negative isolates.

	E. coli	K. pneumoni	P. mirabilis	P. aeruginosa	S. paucimobilis	E. cloacae complex	A. baumannii	S. marcescens	Salmonella group	E. aerogenes	K. oxytoca	A. haemolyticus	A. lwoffi	M. morganii	Shigella group
Ampicillin	929 (67.7)	250 (74.9)	46 (59.7)	20 (29.9)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	7 (58.3)	0 (0)	0 (0)	5 (55.6)	1 (33.3)
Amoxicillin/Clavulanate	190 (13.8)	46 (13.8)	7 (9.1)	21 (31.3)	0 (0)	19 (70.4)	0 (0)	2 (40)	0 (0)	18 (72)	1 (8.3)	0 (0)	0 (0)	5 (55.6)	1 (33.3)
Amikacin	3 (0.2)	9 (2.7)	2 (2.6)	2 (3)	0 (0)	1 (3.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)
Aztreonam	81 (5.9)	23 (6.9)	1 (1.3)	0 (0)	0 (0)	1 (3.7)	0 (0)	0 (0)	0 (0)	2 (8)	2 (16.7)	0 (0)	0 (0)	0 (0)	1 (33.3)
Ceftazidime	343 (25)	111 (33.2)	3 (3.9)	7 (10.4)	0 (0)	13 (48.1)	4 (30.8)	0 (0)	0 (0)	11 (44)	2 (16.7)	0 (0)	0 (0)	1 (11.1)	2 (66.7)
Cefixime	431 (31.4)	105 (31.4)	6 (7.8)	21 (31.3)	1 (25)	13 (48.1)	0 (0)	1 (20)	0 (0)	13 (52)	0 (0)	0 (0)	0 (0)	1 (11.1)	0 (0)
Ciprofloxacin	364 (26.5)	31 (9.3)	8 (10.4)	4 (6)	1 (25)	1 (3.7)	4 (30.8)	0 (0)	0 (0)	1 (4)	2 (16.7)	1 (25)	1 (20)	1 (11.1)	1 (33.3)
Ceftriaxone	449 (32.7)	112 (33.5)	2 (2.6)	12 (17.9)	0 (0)	10 (37)	2 (15.4)	1 (20)	0 (0)	15 (60)	1 (33.3)	1 (25)	0 (0)	1 (11.1)	1 (33.3)
Cefotaxime	111 (8.1)	36 (10.8)	1 (1.3)	4 (6)	0 (0)	3 (11.1)	2 (15.4)	0 (0)	0 (0)	2 (8)	2 (16.7)	0 (0)	0 (0)	0 (0)	0 (0)
Ertapenem	6 (0.4)	3 (0.9)	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefepime	55 (4)	15 (4.5)	2 (2.6)	2 (3)	0 (0)	1 (3.7)	1 (7.7)	0 (0)	0 (0)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)
Nitrofurantoin	29 (2.1)	26 (7.8)	48 (62.3)	17 (25.4)	0 (0)	0 (0)	1 (7.7)	2 (40)	1 (33.3)	3 (12)	0 (0)	0 (0)	0 (0)	3 (33.3)	0 (0)
Gentamicin	193 (14.1)	79 (23.7)	14 (18.2)	3 (4.5)	0 (0)	9 (33.3)	4 (30.8)	0 (0)	0 (0)	7 (28)	1 (8.3)	1 (25)	0 (0)	2 (22.2)	0 (0)
Imipenem	5 (0.4)	2 (0.6)	7 (9.1)	2 (3)	0 (0)	1 (3.7)	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (22.2)	0 (0)
Levofloxacin	46 (3.4)	5 (1.5)	0 (0)	2 (3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Meropenem	4 (0.3)	2 (0.6)	0 (0)	1 (1.5)	0 (0)	0 (0)	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
TMP/SMX	714 (52)	156 (46.7)	43 (55.8)	20 (29.9)	1 (25)	14 (51.9)	2 (15.4)	0 (0)	0 (0)	9 (36)	4 (33.3)	1 (25)	0 (0)	6 (66.7)	1 (33.3)
Piperacillin/tazobactam	67 (4.9)	11 (3.3)	0 (0)	6 (9)	0 (0)	2 (7.4)	4 (30.8)	0 (0)	0 (0)	6 (24)	2 (16.7)	1 (25)	0 (0)	0 (0)	1 (33.3)

4. DISCUSSION AND CONCLUSION

The problem of antimicrobial resistance in bacterial pathogens is described as a growing global crisis. The reported resistance to common pathogens in many parts of the world has reached a level where the experimental use of many of the strongest and most reliable antimicrobial agents available has been ineffective. (Martens E and Demai, (2017)) The present study is unique because no research has been conducted with this large sample size in Erbil. The contamination rate is low as 2.8 % and there is no difference between age groups. This is compatible with literature. The urinary tract pathogens were determined higher in females in all age groups which is also as expected. (Flores-Mireles AL, (2015)) (TM, (2012)) In general, the level of antimicrobial resistance among gram-negative uropathogens in Erbil is relatively high, which is a warning to prevent unnecessary use of antibiotics. Also in the present study, compared to others, the ratio of E. coli to other pathogens was higher. Therefore, the prevalence of E. coli is increasing compared to previous studies in Erbil.

Findings from previous studies have shown that resistance to antibiotics varies in different parts of the world. Developing countries such as India and Libya have reported very high resistance to conventional antibiotics (Ganguly N, (2011)). However, in the present study, antibiotic resistance was relatively high and it seems that widespread and irrational use of antibiotics has caused this resistance. If available, planning of treatments depending on antimicrobial susceptibility testing results is a logical approach to prevent increased antimicrobial resistance. If antimicrobial susceptibility testing is not available, empirical treatment schemes should be designed with local data such as in this study to avoid the treatment failures with unuseful antibiotics.

In the present study, the most common uropathogen is E. coli, which accounted for 70% of gram-negative isolates, followed by K. pneumonia (17%), Proteus mirabilis (3.9%) and P. aeruginosa (3.4%). Previous studies have reported similar results. In the study of Ahmed et al. (2019), the most common uropathogens were E. coli (27%), K. pneumoniae (12.4%), Proteus mirabilis (4.5%) and P. aeruginosa (4.5%) (Ahmed SS, (2019)). Aktaş and Denktaş (2017) identified E. coli as the most common strain (48%) among gram-negative pathogens, followed by Klebsiella. (E, (2017)) Abujnah et al. (2015) reported similar results in Libya. Osman (2019) Gupta et al. (2007) and Giwa et al. (2018) identified the most common gram-negative uropathogens as E. coli, K. pneumoniae, P. mirabilis and P. aeruginosa (Giwa FJ, (2018)) (Gupta N, (2007)) (AA., (2019)) (Abujnah AA, (2015))) The present study evaluated a larger sample than others, which increases the validity of the results.

In the present study, imipenem, meropenem, ertapenem and amikacin were the most effective antibiotics against gram-negative uropathogens (especially Escherichia coli). In the study of Aktaş and Denktaş (2020), E. coli strains showed the lowest resistance to these four antibiotics (E, (2017)) A study by Ali et al. (2017) in Erbil showed the lowest resistance of Escherichia coli to imipenem (1.25%), meropenem and amikacin (1.9%) and Ertapenem (3.8%). (Ali FA, (2017)) These findings were consistent with our results. On the other hand, in the present study, E. coli strains showed the highest resistance to Ampicillin (67.7%), TMP / SXT (52%), Ceftriaxone (32.7%) and Cefixime (31.4%). Consistent with these findings, Edlin et al. Showed that E.

coli strains in UTI patients were most resistant to ampicillin and trimethoprim / sulfamethoxazole (Edlin RS, (2013)). A study by Abujnah et al. (2015) in Libya showed that the resistance to ampicillin in Escherichia coli and Klebsiella species was 69.2% and 100%, respectively (Abujnah AA, (2015)). Similarly, the study of Osman (2019) and Mohammed et al. (2016) reported the highest antimicrobial resistance to ampicillin. (AA., (2019)) (Abujnah AA, (2015)) (Mohammed, (2016))) Gupta et al. (2007) reported resistance of co-trimoxazole, ampicillin and ciprofloxacin to the three uropathogens of E. coli, K. pneumoniae and Pseudomonas between 90% to 96%, 92% to 98% and 55% to 65%, respectively. (Gupta N, (2007))

ESBL-producing bacteria are resistant to many common antibiotics, and the increasing prevalence of these bacteria is an indicator of increased antimicrobial resistance. In the present study, 38.6% of E. coli strains were ESBL positive. Aktaş and Denktaş (2020) reported 27.8% of E. coli samples as extended-spectrum beta-lactamase-producing strains. (E, (2017)) Given the novelty of the above study, it can be concluded that the prevalence of EBSL-producing strains of E. coli in Erbil, Iraq is higher than in Turkey. It appears to be because of the unscrupulous use of antibiotics in Erbil. According to previous studies, the treatment of ESBL-producing E. coli, which is commonly observed in community-acquired UTIs, is a challenge. (Hertz FB, (2016)) In the study of Giwa et al. (2018), ESBL-producing strains generally accounted for 34.3% of cases. (Giwa FJ, (2018)) These findings were consistent with the results of present study with a 35.5% prevalence of ESBL-positive bacteria.

The most common gram-negative urinary pathogen determined in this study was E. coli. The highest resistances of gram-negative urinary pathogens were against the antibiotic ampicillin, trimethoprim/sulfamethoxazole and ceftriaxone, and the lowest resistances were for amikacin, meropenem, ertapenem and imipenem. The results of this study also show that Gram-negative uropathogens show significant resistance to trimethoprim/Sulfamethoxazole and ciprofloxacin, which are uncomplicated first-line therapies for UTI, and are unlikely to be ineffective. One of the best options for antibiotic treatment is fluoroquinolones, that show significantly low resistance levels. The data presented in this study, which was collected over a long period of time and had a large sample size, can be useful for planning empirical treatment schemes and setting appropriate and rational antibiotic use policies.

References

AA., O. ((2019)). Antibiotic Resistance of Bacteria isolated in Urinary Tract Infections in Erbil City. Zanco Journal of Pure and Applied Sciences. pp. 31(4), pp.42-49.

Abujnah AA, Z. A.-M. ((2015)). Multidrug resistance and extended-spectrum β-lactamases genes among0 Escherichia coli from patients with urinary tract infections in Northwestern Libya.

Ahmed SS, S. A. ((2019)). Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. International Journal of Health Sciences. pp. 13(2), p.48.

Ali FA, M. E. ((2017)). Antibiotic resistance among Escherichia coli isolated from different clinical samples in Erbil City. International Journal of Research Studies in Science, Engineering and Technology. pp. 4(10), pp.12-21.

Anderson, G. G. ((2010)). Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. Infection and immunity. pp. 78(3), 963-975.

Beyer, A. K. ((2019)). Validity of microscopy for diagnosing urinary tract infection in general practice–a systematic review. Scandinavian Journal of Primary Health Care. pp. 37(3), 373-379.

E, A. O. ((2017)). Five-Year Evaluation of the Urine Culture Results and Antimicrobial Susceptibility Profiles of Isolated E. coli Strains. . pp. 4(9), pp.18-21.

Edlin RS, S. D. ((2013)). Antibiotic resistance patterns of outpatient pediatric urinary tract

infections. The Journal of urology. pp. 190(1), pp.222-227.

Flores-Mireles AL, W. J. ((2015)). Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology, . pp. 13(5), pp.269-284.

Ganguly N, W. C. ((2011)). Situation Analysis Antibiotic Use and Resistance in India. Public Health Foundation of India, and Center for Disease Dynamics, Economics and Policy. pp. 209-212.

Giwa FJ, I. O. ((2018)). Extended-Spectrum beta-lactamase production and antimicrobial susceptibility pattern of uropathogens in a Tertiary Hospital in Northwestern Nigeria. Annals of Tropical Pathology. p. 9(1).

Gupta N, K. S. ((2007)). Antimicrobial susceptibility of uropathogens in India. J Infect Dis Antimicrob Agents. pp. 24(1), pp.13-18.

Hertz FB, S. K.-O.-M. ((2016)). Epidemiological factors associated with ESBL-and non ESBL-producing E. coli causing urinary tract infection in general practice. Infectious Diseases.

Köves, B. C. ((2017)). Benefits and harms of treatment of asymptomatic bacteriuria: a systematic review and meta-analysis by the European Association of Urology Urological Infection Guidelines Panel. pp. 72(6), 865-868.

Martens E and Demai, A. ((2017)). The antibiotic resistance crisis, with a focus on the United States. The Journal of antibiotics. pp. 70(5), pp.520-526.

Mohammed, M. A. ((2016)). "Prevalence and antimicrobial resistance pattern of bacterial strains isolated from patients with urinary tract infection in Messalata Central Hospital, Libya." Asian Pacific journal of tropical medicine . pp. 771-776.

Oteo, J. P.-V. ((2010).). Extended-spectrum β-lactamase producing Escherichia coli: changing epidemiology and clinical impact. Current opinion in infectious diseases. pp. 23(4), 320-326.

Scholes, D. H. ((2000)). Risk factors for recurrent urinary tract infection in young women. The Journal of infectious diseases. pp. 182(4), 1177-1182.

T, W. C. ((2017)). The pathophysiology of urinary tract infections. Surgery (Oxford), . pp. 35(6), pp.293-298.

TM, H. ((2012)). Uncomplicated urinary tract infection. New England Journal of Medicine. pp. 366(11), pp.1028-1037.