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RESEARCH ARTICLE

STUDY SOME PATHOGENCITY EFFECT OF *PROVIDENCIA RETTGERI* ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS

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ABSTRACT

From April to December 2014 Four hundred and ninty mid stream urin specimens were collected from patients suffering from urinary tract infections and from urinary catheterized patients in Baquba Teaching Hospital and Al -Betul Hospital, and Baghdad Teaching Hospital. Out of 14 Providencia isolates, 9 isolates were diagnosed as *Providencia rettgeri*. This identification was confirmed by biochemical tests, Api 20 E system and vitek 2 compact system. The results of susceptibility of isolates to antibiotics were tested, using disc diffusion method showed the bacterial isolates have complete resistance for Ampicillin, Tetracyclin,Vancomycin with a percentage of 100%, the results indicate that the antibiotics Amikacin and imipeneme were found to be the most effective drugs against the strains under the study i.e. the resistance to these antibiotics were 0%. All strains of *P. rettgeri* isolates were tested to ability to biofilm production, by two methods TCP and CRA , out of nine isolates only 4(44.5%) and 1(11.2%) were found tobe strong biofilm producer by CRA and TCP, respectively. The histological section of mice (kidney and bladder) injected ineraperitonial with two groups of *P. rettgeri* isolates, group (A) injected mice with *P. rettgeri* P9 isolate (strong biofilm producer and multi drug resistant especially beta – lactam) and group (B) injected mice with *P.rettgeri* P7 isolate(weak or non biofilm producer and sensitive to different antibiotics especially beta-lactam) , results showed Several pathological changes have been caused by *P.rettgeri* isolates in mice represented by sever inflammatory cell infiltration with congestion and degeneration with necrosis of epithelial mucosal layer in urinary bladder, while the Kidney suffered from sever inflammatory cells infiltration of PMN into Glomerulus and deposition of red fibrinoid material within glomerulus capillaries with necrosis of epithelial renal tubules. It was clear that the strong producer isolate *P. rettgeri* P9 was more virulent than the weak(non producer producing isolate *P.rettgeri* P7.

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INTRODUCTION

Providenciae rettgeri is is a species of gram-negative bacteria that is closely related to members of both the *Proteus* and *Morganella* genera (O'Hara *et al*., 2000). It is a faculative anerobe, and is fairly ubiquitous across a wide range of environments. *P. rettgeri* is known mainly for its association in the gut microbiome with humans and insects, and can potentially be the cause of oppurtunistic infections among these species. In humans, *P. rettgeri* has been implicated in urinary tract infection, traveler's diarrhea and a variety of noscomial infection-related disesases (Yoh *et al.,* 2005). *P. rettgeri* may form a biofilm, which provides specific adherence characteristics allowing for its persistence in the catheterized urinary tract and catheter encrustation (Stickler and Zima off, 1994).

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An indwelling urinary catheter allows a biofilm to form on the cathether's surface, promoting bacterial growth and marks the start of a potentially fatal infection (Rahav *et al*., 1994; Shiroto *et al*., 2005). The ability of *P. rettgeri* to produce urease enables it to thrive in these conditions. *P. rettgeri* produce urease which generates ammonia. This elevates the pH of urine and leads to both calcium and magnesium phosphate deposits in both the urine and on the biofilm itself (Choong *et al*., 2001; Broomfield *et al*., 2009). The urinary tract of the compromised or catheterized patient is the most common site of *P. rettgeri* and *P. stuartii* infections, the rise in importance of these two species is associated with their tendency to cause nosocomial infections and with their marked resistance to numerous antibiotics (Jones and Mobley, 1987; Shiroto *et al*., 2005; Manos and Belas, 2006; Lee *et al*., 2007). *P. stuartii* and *P. rettgeri* are the most common *Providencia* species that cause human infection. While uncommon in most clinical settings, these organisms tend to cause cystitis in patients with bladder catheters and are primarily associated

with complicated urinary tract infections (Rahav *et al.,* 1994; Nicoll, 2001).

MATERIALS AND METHODS

Isolation and identification

From April to December 2014 Four hundred and ninty mid stream urin specimens were collected from patients suffering from urinary tract infections and from urinary catheterized patients in Baquba Teaching Hospital and Al -Betul Hospital, and Baghdad Teaching Hospital. All specimens were cultured on MacConkey agar (Bioanalyse/ Turkey) cultivated at 37 °C for 24 h. Lactose non fermentor colonies were transferred to nutrient agar (Oxoid, England), identified in accordance to (Macfaddin, 2000), by conducting necessary biochemical tests , in addition to api 20 E system and vitek 2compact system. The ability of *P. rettgeri* strains to produce hemolysin; urease, phenylalanine (17) was investigated.

Antibiotic Susceptibility Test

Antibiotic susceptibility test of nine *P.rettgeri* isolates was done on Mueller Hinton agar using the following antibiotic discs: Amoxicillin, Ampicillin, Amikacin, Aztreonam, Cephalexin, Ceftazidime, Cefepime, Cefotaxime, Ciprofloxacin, Clindamycin, Erythromycin, Gentamicin, Imipenem, Meropenem, Nalidixic acid, Penicillin G, Tetracyclin and Vancomycin

Adhesion test and Biofilm Formation

The ability of *P. rettgeri* isolates to produce slime layer and biofilm formation were applied on nine isolates and were tested by two methods; Congo red agar (CRA) method **(**Freeman *et al*., 1989; Mathur *et al*., 2006), a positive result was indicated by black colonies with a dry crystalline consistency. Non- slime produces usually remained pink. An indeterminate result was indicated by a darkening of the colonies but with the absence of a dry crystalline colonial morphology and Tissue culture plate method (TCP),according to the method described by Amaral *et al*. in (2005) was followed with some modifications. Briefly, overnight cultures were diluted (1:200) with TSB supplemented with 1% (w/v) glucose. Aliquots (200 µl) from the culture were then transferred to the wells of a 96-well polystyrene microtiter plate (Corning, Inc., Corning, N.Y.), and later incubated overnight at 37°C. After incubation, supernatants were removed from each well and biofilms were gently washed twice with normal saline, then dried and fixed at 65°C for 1 hr.

Subsequently, the plates were stained with 0.1% (w/v) methylene blue for 10 min, gently washed twice and the quantitative analysis of biofilm production was performed by adding 200 µl of 95% ethanol for 10 min. Finally, 200µl from each well was transferred to a new microtiter plates and the absorbance of the methylene blue present in the destaining solution (ethanol) was measured at 580 nm by microplate reader

Animals

Female mice (*Mus musculus)*, aged 8 weeks, weighing 25–30 gm were divided into 3 groups (A,B and C), three mice per group. Strong Biofilm producer *P. rettgeri P9* strain was injected interaperitonial in A group concentration 10^8 cfu / ml. Group B were injected with (weak) non producer *P. rettgeri P7* using the same concentration mentioned earlier. While group C was injected with phosphate buffer saline (PBS) and served as a control group. Four days later. Animals were killed; bladders and kidneys were removed aseptically for histopathological study. The tissues sections were prepared according to Humason (1972). The histopathological changes were observed by Dr. Salem Al-uobiadi under the magnification power 200 X of light microscope.

RESULTS AND DISCUSSION

Results showed, 445 (90.82%) specimens have positive growth on isolating media. Fourteen (2.85) isolates were identified as Providencia *spp.* and 431(87.95%) isolates were identified as other gram negative bacteria, while 45(9.18%) specimens were showed no growth of microorganisms from total specimens (490). All the isolates of *P. rettgeri* were tested for their ability to produce slime layer by the Congo red agar method to choose the isolates that have a greater ability for adhesion. The positive results showed 100% of *P. rettgeri* isolates produced slime layer indicated by formation of black colonies with dry crystalline consistency. method described here are based on the enhancement of exopolysacharide production by using enriched media, Congo red agar method requires the use of a highly nutritious medium Brain Heart Infusion broth with 5% sucrose supplementation. Congo red stain was chosen because it has been used as a stain for showing the presence of the exopolysaccharide of aquatic gram negative bacilli (Freeman *et al.*, 1989). This method correlated well with TCP for identifying strong biofilm producers. We have performed the TCP method by addition of 1% glucose in trypticase soy broth. Addition of sugar helps in biofilm formation. (Kim, 2001). This was also reported by study conducted by Mathur *et al*. (2006).

After reading the absorbance at 590 nm, a variation in biofilm formation among the nine investigated *P.rettgeri* isolates was observed, as suggested by the recorded absorbances. Previous studies documented that the optimal conditions for biofilm formation (i.e., growth medium, temperature and time of incubation) must be determined empirically for each microbe (Cabral *et al*., 2011; O'Toole, 2011. Thus, these results might explain, at least in part, the marked persistence of *P rettgeri* in hospitals and their involvement in nosocomial infections (Rodriguez-Baño *et al*., 2008). By CRA method, the number of strong biofilm producers were 4(44.44%), moderate were 5(55.55%) and non isolate were weak or non-biofilm producers were. Very different results were observed by the CRA method, with which only four isolates showed black colonies with crystalline appearance, While, among nine isolates, TCP, the standard method, detected 1(11.11%) as strong, 5(55.55%) as moderate and 3(33.33%) as weakly biofilm producers Table (1).

Table 1. Result of different method for biofilm producing *P. rettgeri* isolates

Level of biofilm production	NO. of biofilm producing isolates (%) using different method	
	TCP	CRA
High producer	1(11.11%)	4(44.44%)
Moderate producer	5(55.55%)	5(55.55%)
Weak/non producer	3(33.33%)	_
Total	9(100%)	9(100%)

TCP= Tissue Culture Plate; CRA=Congo Red Agar

Histopathological changes

The microscopic examination of stained tissue sections of organs(Kidney and Bladder) obtained from dead mice after (4days) injected with interaperitonial of two groups of *P. rettgeri*, group 1 (*P. rettgeri* P9 strong biofilm producer and multidrug resistance) showed sever changes in kidney and bladder induced by bacterial suspension, and group 2 (*P. rettgeri* P7 weak or non producer biofilm and sensitive to many antibiotics espacially beta-lactam) that showed mild changes in kidney and bladder, These changes compared with control animals that injected with PBS as negative controls. The histological section of the urinary bladder of mice injected with life bacteria (*P. rettgeri* P9) showed sever inflammatory cell infiltration with congestion and degeneration with necrosis of epithelial mucosal layer (Figure 2.). While in the urinary bladder of mice injected with (*P. rettgeri* P7) showed mild inflammatory reaction in the wall of urinary bladder with degeneration of epithelial cells (Figure 3). This section of a normal histological structure of control urinary bladder in the sub mucosa as showed in Figure (1).

The microscopic examination of stained tissue sections showed that the histopathological defects of kidney of mice injected with live bacteria (*P. rettgeri* P9) included sever inflammatory cells infiltration of PMN into Glomerulus and deposition of red fibrinoid material within glomerulus capillaries with necrosis of epithelial renal tubules (Figure 3) Wherase injected mice with (*P.rettgeri* P7) section showing necrosis of epithelial renal tubules and inflammatory cells infiltration (Figure 3). This results agreement with study by Hayder and Frias, (2013) were showed in their study sever histopathological changes in kidney and bladder induced by bacterial suspension than LPS, included the congestion, hemorrhage, migration and infiltration of inflammatory cells, tissue necrosis and oedema but in spleen there were hypertrophy of white bulb and congestion of red bulb.

This result was converged with study of Lloyd *et al*. (2009) who demonstrated that focal infiltration of inflammatory cells, in the pelvis of kidney and moderate, widespread edema and minimal inflammatory infiltrate in bladder. Recruitment of neutrophils, shown to be essential for clearance of bacteriafrom the bladder and kidneys (Haraoka *et al*., 1999). The presence of pyelonephritis, or inflammation of the renal pelvis, was determined based upon the number of inflammatory foci (neutrophils) present in the renal pelvis, and the severity of inflammation was determined by the number of neutrophils in the tissues directly surrounding the pelvis (peripelvic region) (Lloyd *et al*., 2009).

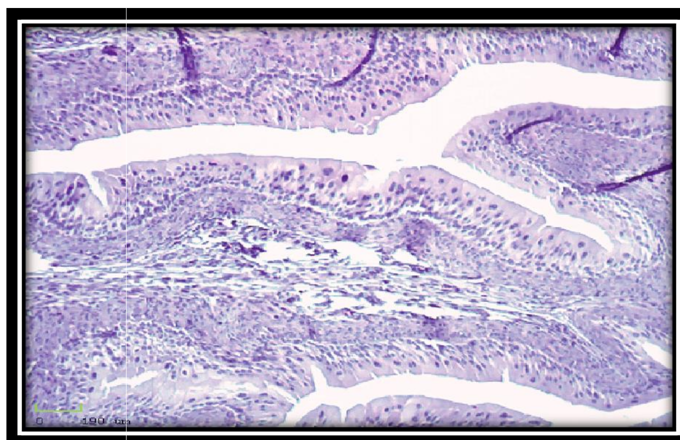

Figure 1. Section of urinary bladder of control (X200) (H and E)

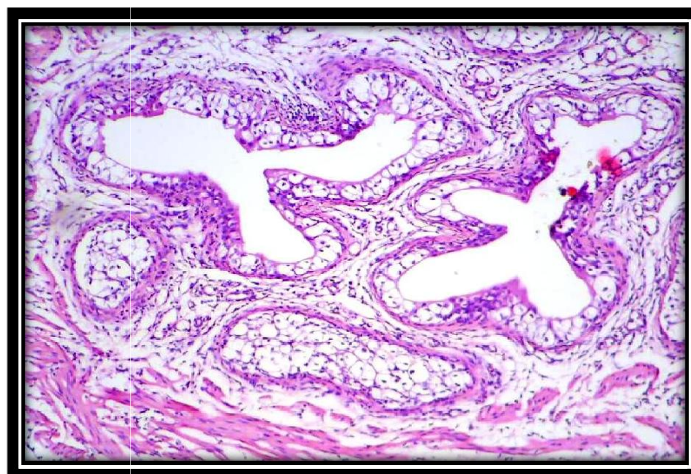

Figure 2. The histological section of the urinary bladder of mice injected with life bacteria (*P. rettgeri* P7) showed mild inflammatory reaction in the wall of urinary bladder with degeneration of epithelial cells (X200) (H&E)

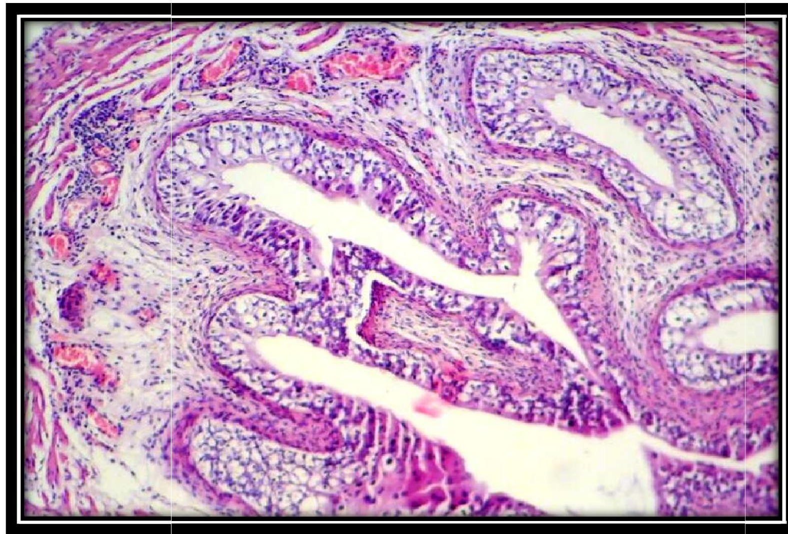

Figure 3. The histological section of the urinary bladder of mice injected with life bacteria (*P. rettgeri* P9) showed sever inflammatory cell infiltration with congestion and degeneration with necrosis of epithelial mucosal layer (X200) (H&E stain)

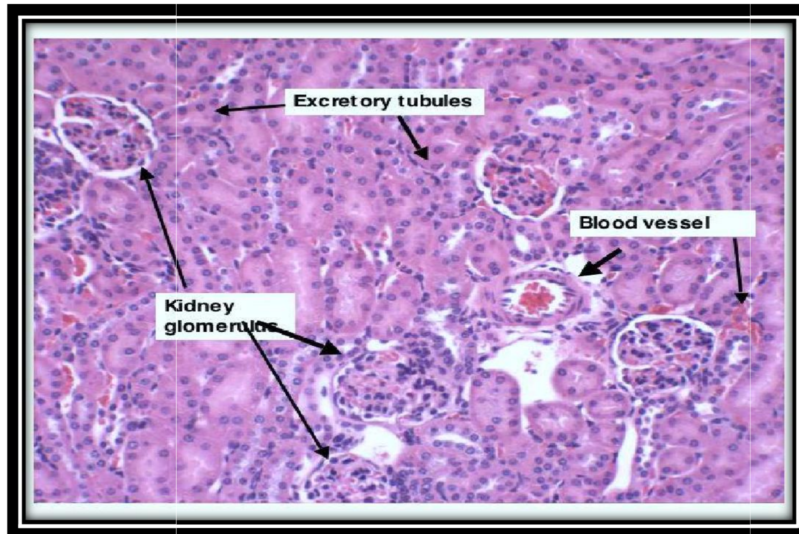

Figure 4. Section of Kidney of control mouse (X200) (H&E stain)

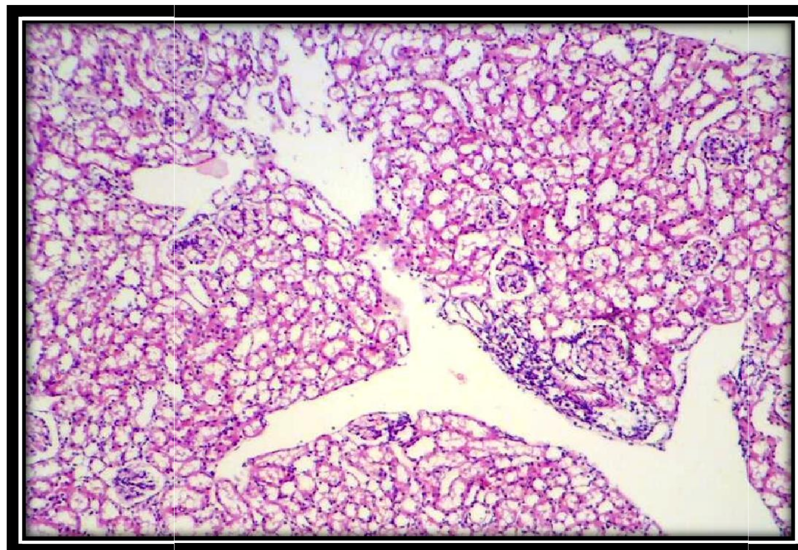

Figure 5. The microscopic examination of stained tissue sections showed that the histopathological defects of kidney of mice injected with live bacteria(*P. rettgeri* P9) included sever inflammatory cells infiltration of PMN into Glomerulus and deposition of red fibrinoid material within glomerulus capillaries with necrosis of epithelial renal tubules(X200) (H&E stain)

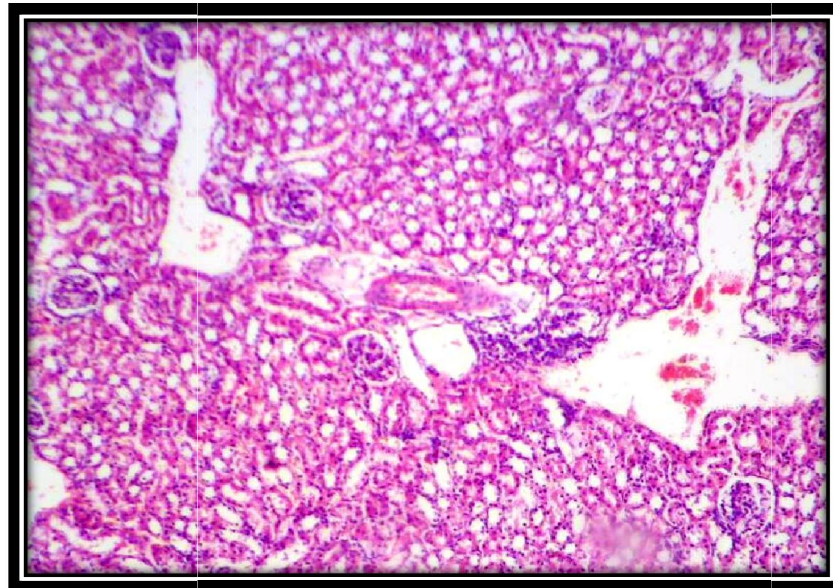

Figure 6. The microscopic examination of stained tissue sections showed that the histopathological defects of kidney of mice injected with live bacteria(*P. rettgeri* P7) section showing necrosis of epithelial renal tubules and inflammatory cells infiltration.(X200)(H&E stain)

REFERENCES

Broomfield, R. J., Morgan, S. D., Khan, A. and Stickler, D. J. 2009. Crystalline bacterial biofilm formation on urinary catheters by urease producing urinary tract pathogens: a simple method of control. *Journal of Medical Microbiology,* 58(10): 1367–75-

Choong, S., Wood, S. and Whitfield, H. 2001. Catheter associated urinary tract infection and encrustation. *Int. J. Antimicrob. Agents*, 17:305-310.

Freeman, D.J., Falkiner, F.R. and Keane, C.T. 1989. New method for detecting slime production by coagulase negative *stap hylococci. J. Clin. Pathol.,* 42:872-874.

Haraoka, M., Hang, L., Frendeus, B., Godaly, G., Burdick, M., Strieter, R. and Svanborg, C. 1999. Neutrophil recruitment and resistance to urinary tract infection. *J. Infect. Dis., 180*:1220–1229.

Hayder, S.O. and Frias, G.A. 2013. Pathogenesis of *Providencia rettgeri* in mice. *Journal of Babylon University/Pure and Applied Sciences*, 8(21):2785-2800.

Humason, C.L. 1972. Animal tissue techniques, 3rd edition. W.H Freeman and company.p:641

Jones, B.D. and Mobley, H. L. T. 1987. Genetic and Biochemical Diversity of Ureases of *Proteus*, *Providencia*, and *Morganella* Species Isolated from Urinary Tract Infection. *Infec. and Immu.,* 55 (9): 2198-2203.

Lee, H.W., Kang, H.Y., Shin, K.S. and Kim, J. 2007. Multidrug-resistant *Providencia* isolates carrying $bla_{\text{PER-1}}$, $bla_{\text{VIM-2}}$, and *arm A. J. Microbiol.,* 45:272-274.

Lloyd, A.L., Smith, S.N., Eaton, K.A. and Mobley, H.L.T. 2009. Uropathogenic *Escherichia coli* Suppresses the Host Inflammatory Response via Pathogenicity Island Genes sis A and sis B. Infect. and Immu, 77(12): 5322–5333.

Manos, J. and Belas, R. 2006. The Genera *Proteus*, *Providencia*, and *Morganella*. Prokaryotes, 6:245–269.

Mathur, S. 2006. Factors affecting crystal preciptation from urine individuals with long term urinary catheters colonized with urease positive bacterial species. Urol Res, 34:173-177.

Nicolle, L.E. 2001. Urinary tract pathogens in complicated infection and in elderly individuals. *J. Infect Dis.,* 183(11):5-8.

O'Hara, C.M., Brenner, F. and Miller, H. 2000. Classification, identification, and clinical significance of *Proteus, Providencia*, and *Morganella*. Clin. Microb. Rev, 13:534-546.

Rahav, G., Pinco, E., Silbaq, F. and Bercovier, H. 1994. Molecular epidemiology of catheter-associated bacteriuria in nursing home patients. *Journal of Clinical Microbiology*, 32(4): 1031–4.

Shiroto, K., Ishii, Y., Kimura, S., Alba, J., Watanabe, K., Matsushima, Y. and Yamaguchi, K. 2005. Metallo-beta-lactamase *IMP-1* in *Providencia rettgeri* from two different hospitals in Japan. *J. Med Microbiol.,* 54:1065-1070.

Stickler, D. J. and Zimakoff, J. 1994. Complications of urinary tract infections associated with devices for long-term bladder management. *J. Hosp Infect.,* 28: 177–194

Yoh, M., Matsuyama, J., Ohnishi, M., Takagi, K., Miyagi, H., Mori, K. and Park, K.S. 2005. Importance of *Providencia* species as a major cause of travellers' diarrhoea. *Journal of Medical Microbiology,* 54 (11): 1077–82.

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