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Research Article

EFFECT OF PH ON THE ANTIMICROBIAL SUSCEPTIBILITY OF PLANKTONIC-GROWN PSEUDOMONAS AERUGINOSA, ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS ATCC STRAINS

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ABSTRACT

The *in vitro* susceptibilities of bacteria to antibiotic are traditionally tested in media with a neutral pH. However, in a living organism and particularly in humans, the acidity varies a lot from one place to another. The present study aims to evaluate influence of pH in the expression of *in vitro* antimicrobial susceptibility among planktonic-grown *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* ATCC® strains. Results show that the pH value affect antimicrobial activity differently, depending upon the strain used. Indeed, *P. aeruginosa and E. coli* are more susceptible to tetracycline under acidic and neutral pH condition whereas *S. aureus* is equally susceptible to tetracycline in all tested pH condition. Conversely, *P. aeruginosa, E. coli and S. aureus* are more susceptible to erythromycin, kanamycin and gentamycin under neutral and alkaline pH condition. This preliminary study highlights that pH parameter should be considered in evaluation of antimicrobial effectiveness and this is a point that should be considered by antibiotic prescriber in delivering antibiotic prescription.

KEYWORDS: Antimicrobial susceptibility, pH, *Pseudomonas, Staphylococcus, Escherichia.*

INTRODUCTION

The *in vitro* susceptibilities of bacteria to antibiotic are traditionally tested in media with a neutral pH. However, in a living organism and particularly in humans, the acidity varies a lot from one place to another. For instance, acidity all along the digestive system has been measured from pH 2 to 8 (Fallingborg, 1999)[1], likewise pH is around 7.32 to 7.42 in the blood (Waugh and Grant, 2007)[2] and around 5 on the skin (Lambers *et al.,* 2006)[3]. Moreover, the variation of growth medium quality and its pH have been reported elsewhere to impact on the antimicrobial susceptibility of bacteria (Falagas *et al.,* 1997)[4]. Indeed, The role of an acidic pH and low oxygen tension in decreasing the *in vitro* susceptibilities of several bacterial species to aminoglycosides (Bryant *et al.,* 1992)[5] and in reducing the efficacy of aminoglycosides in the treatment of intra-abdominal infections have been demonstrated elsewhere (Simmens *et al.,* 1993)[6]. Thus, this phenomenon may influence on *in vivo* effectiveness of antimicrobial used to treat infection and beyond pharmacodynamics and pharmacokinetic properties of antimicrobial, the effectiveness of antimicrobial may depends on the localization of infection. Accordingly, it is useful to know the conditions that optimize action of these antibiotics which would not only guide judiciously treatment depending on the site of infection but also avoid therapeutic failures that may be the source of future resistance to antibiotics. Thus,

the present study aims to evaluate influence of pH in the expression of *in vitro* antimicrobial susceptibility among planktonic-grown *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* ATCC® strains

MATERIALS AND METHODS

Bacterial strains, culture conditions and antimicrobial drugs

Reference strains *Escherichia coli* ATCC® 25922, *Staphylococcus aureus* ATCC® 25923 and *P. aeruginosa* ATCC® 27853 were purchased from OXOID and grown in LB-MOPS broth. LB-MOPS broth has been adjusted at 4 different pH (5, 6, 7, 8). Antimicrobial drugs (tetracycline, erythromycin, gentamycin and kanamycin) have been selected according to their availability and were purchased from TCI® (Tokyo chemical industry Co. LTD, Japan) and dissolved in deionized water.

Antibacterial Assay

For the determination of MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration), *S. aureus* ATCC® 25923, *E. coli* ATCC® 25922 and *P. aeruginosa* ATCC® 27853 were grown on 96-well micro-plates with 150 µl of LB broth in the presence of each antimicrobial drugs at different concentrations (0.25 to 1024 µg/ml) and incubated at 37°C for 24 h. The MIC was defined as the lowest concentration that completely inhibited growth as detected by the naked eye (Chérigo *et al.,* 2009)[7]. All inhibited growth culture were then sub-cultured onto LB agar plate and incubated at 37°C for 24 h to determine the MBC which was defined as the lowest concentration that yielded negative sub-cultures (Okusa *et al.,* 2007)[8]. Antibiotic is considered to be bacteriostatic if MBC/MIC>4 and bactericide if MBC/MIC≤4. Determination of resistance has been defined by the EUCAST or CASFM resistance breakpoint if available and MIC under EUCAST/CASFM resistance breakpoint has been considered as susceptible (EUCAST, 2017; CASFM, 2015)[9, 10].

RESULTS

P. aeruginosa is more susceptible to tetracycline under acidic and neutral pH condition with optimal MIC value at pH8. Conversely, *P. aeruginosa* is more susceptible to erythromycin, kanamycin and gentamycin under neutral and alkalin pH condition (Table 1). Moreover, effectiveness of erythromycin seems to be drastically affected by acidic pH where MIC value is largely out of MIC recorded by Fass et Barnishan, 1979 [11] (512 versus 64µg/ml). Tetracycline and erythromycin are bacteriostatic antibiotic in concordance with literature whereas kanamycin and gentamycin are bactericidal agents. Comparing to the MIC target of EUCAST *P. aeruginosa* is susceptible to gentamycin only at neutral and alkaline pH (Table 2). *P. aeruginosa* is naturally resistant to tetracycline, erythromycin and kanamycin so that these antibiotics are not used in therapeutic against *P. aeruginos*.

Table 1: MIC and MBC of tetracycline, erythromycin, kanamycin and gentamycin against *Pseudomonas aureginosa ATCC*® 27853, *Escherichia coli ATCC*® 25922 and *Staphylococcus aureus ATCC*® 25923 grown in LB medium according to pH variation.

		Tetracycline (µg/ml)		Erythromycin (µg/ml)		Kanamycin (µg/ml)		Gentamycin (µg/ml)	
		MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
P. aeruginosa	pH 5	8	512	512	>1024	32	64	32	64
	pH 6	4	256	256	>1024	16	32	32	32
	pH 7	4	256	64	>1024	8	16	4	8
	pH 8	2	128	8	1024	16	32	2	4
E. coli	pH 5	0.5	2	64	128	1	2	1	1
	pH 6	0.5	2	64	128	0.5	2	1	1
	pH 7	1	4	4	8	0.5	1	0.5	1
	pH 8	8	16	1	2	0.5	1	0.25	1
S. aureus	pH 5	1	4	16	32	8	16	16	16
	pH 6	1	4	4	8	4	16	8	32
	pH 7	1	4	0.25	2	4	16	0.25	2
	pH 8	1	8	1	4	2	4	0.25	1

*bold: lowest MIC and MBC

E. coli is more susceptible to tetracycline under acidic and neutral pH condition with MIC value at pH5-6 whereas alkaline pH seems to increase MIC value. Conversely, *E. coli* is more susceptible to erythromycin, kanamycin and gentamycin under neutral and alkaline pH condition (Table 1). As for *P. aeruginosa,* effectiveness of erythromycin seems to be drastically affected by acidic pH where MIC value is largely out of critical target value recommended by EUCAST or presented by antimicrobial index data (64 versus 1µg/ml). Intriguingly, tetracycline, erythromycin, kanamycin and gentamycin are all bactericidal agent. Comparing to the MIC target of EUCAST *E. coli* is susceptible to tetracycline, kanamycin and gentamycin whatever the pH is. Erythromycin is not used in therapeutic against *E. coli* (Table 2).

Staphylococcus aureus is equally susceptible to tetracycline in all tested pH condition with MIC value at 1 pH6 however with an increased value of MBC at pH8 condition. *Staphylococcus* is more susceptible to erythromycin, kanamycin and gentamycin under neutral and/or alkaline pH condition with at optimal efficiency at pH 7 for erythromycin, pH 8 for kanamycin and both pH7-8 for gentamycin (Table 1). Intriguingly, bacteriostatic or bactericidal profile is also influenced by pH so that an antibiotic may exert bacteriostatic effect in acidic pH and bactericidal in alkaline pH. Tetracycline is bacteriostatic antibiotic in concordance with literature whereas kanamycin erythromycin and gentamycin are bactericidal. Comparing to the MIC target of EUCAST, *Staphylococcus aureus* is susceptible to tetracycline and kanamycin whatever the pH is and susceptible to erythromycin and gentamycin only at neutral and alkaline pH (Table 2).

Table 2: Critical concentration of antibiotic (EUCAST, 2017)

		Tetracycline (µg/ml)		Erythromycin (µg/ml)		Kanamycin (µg/ml)		Gentamycin (µg/ml)	
		MIC	**RB**	**MIC**	**RB**	**MIC**	**RB**	**MIC**	**RR**
P. aeruginosa	pH 5	8	NC	512	NC	32	NC	32(R)	>4
	pH 6	4		256		16		32(R)	
	pH 7	4		64		8		4(S)	
	pH 8	2		8		16		2(S)	
E. coli	pH 5	0.5(S)	>8**	64	NC	1(S)	>16**	1(S)	>4
	pH 6	0.5(S)		64		0.5(S)		1(S)	
	pH 7	1(S)		4		0.5(S)		0.5(S)	
	pH 8	8 (S)		1		0.5(S)		0.25(S)	
S. aureus	pH 5	1(S)	>2	16(R)	>2	8(S)	>16**	16(R)	>1
	pH 6	1(S)		4(R)		4(S)		8(R)	
	pH 7	1(S)		0.25(S)		4(S)		0.25(S)	
	pH 8	1(S)		0.1(S)		2(S)		0.25(S)	

RB: Resistance breakpoints; NC: Resistance breakpoints not communicated by EUCAST and CASFM, *EUCAST Resistance breakpoints; **CASFM Resistance breakpoints; (R): resistant; (S): Susceptible

DISCUSSION

This study indicates that the pH value affect differently effectiveness of antimicrobial, depending upon the strains used. A study has been already demonstrated effect of pH value on the germicidal action of cationic detergents (subtilin A, a surface-active polypeptide antibiotic) *Staphylococcus aureus and Escherichia coli.* Indeed, *Staphylococcus aureus,* are more susceptible to subtilin A as the pH value increases and *Escherichia coli,* are more sensitive as the pH value decreases (Sacks and Pence, 1958) [12]. No explanation has been offered to account for this behavior. Other study demonstrated that acidic pH (5.8 and 6.2) affects the *in vitro* susceptibilities of the *Bacteriodes fragilis* group to several antibiotics (ciprofloxacin and imipenem but the clinical significance of this phenomenon is unclear. However, we should consider that medium used in this study is different than used in other studies that may influence de kinetic growth of bacteria. Thus, comparison between different studies is hazardous.

The effect of pH on the *in vitro* and *in vivo* susceptibilities of bacteria to antibiotics may be mediated through several mechanisms. Firstly, pH influences the permeability of bacteria to antibiotics, as shown in studies of permeability to beta-lactams in *B. fragilis* (cuchural, 1988)[13]. Secondly, the stability and activity of enzymes which inactivate antibiotics are affected by pH, as shown in studies of β-lactamase activity of *B. fragilis* (Nord *et al.,* 1980)[14]. Finally, the stability and kinetics of certain antibiotics are influenced by pH (Smith *et al.,* 1990, Takeuchi *et al.,* 1995)[15, 16]. One parameter that influences permeability of bacteria to antibiotic as well as antibiotic activity is the metabolic activity of bacteria. Indeed, bacteria living in optimal condition of growth increase its metabolic activities through the increase of environment exchange leading to an increased permeability of nutriment and antibiotic. Conversely, under stress condition bacteria decrease the exchange activity and produce a lot of secondary metabolites leading to the development of resistance to antibiotic.

Although it seems evident from our data that acidic pH affects the *in vitro* susceptibilities of the *S aureus, E coli* and *Pseudomonas aeruginosa* to several antibiotics (except for tetracycline), the clinical significance of this phenomenon is unclear. Beyond the proper mode of action, absorption of antibiotic to reach site of action may be influenced by the change in the charge of molecule. Thus, a striking changes in the charge of the molecule over the pH range 4~8 should be also investigated by paper electrophoretic studies. Moreover, future studies should explore the clinical relevance of these laboratory results and the utility of the *in vitro* antimicrobial susceptibility testing of anaerobic bacteria for patients with infections due to anaerobes.

CONCLUSION

This preliminary study highlights that pH parameter should be considered in evaluation of antimicrobial effectiveness and this is a point that should be considered by antibiotic prescriber in delivering antibiotic prescription. Indeed, if an antibiotic such gentamycin is more efficient in pH alkaline, antibiotic consumption should be followed by restricting the ingestion of acidifying foods and conversely, antibiotic consumption should be ingested with acidifying foods if the targeted bacteria are known to be susceptible to a particular antibiotic under acidic pH condition.

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