Journal of Advanced Research in Science and Technology
ISSN: 2352-9989

Influence of temperature on antibiotic susceptibility testing of *Escherichia coli* HO25 by the agar diffusion method

Abdelkrim SAÏD[1,2*] Slimane BENNACER[1,2], Yacine DAOUDI[1,2,3,4], Mohamed KRAIMAT[1,5], Abdelkader HADJ SEYD[6], Michael HOLTZ[7], Noureddine BOURAS[1,8,*], Nasserdine SABAOU[8]

[1]Département de Biologie, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre, Université de Ghardaïa, BP 455, Ghardaïa 47000, Algeria.
[2]Laboratoire Annexe de Contrôle de la Qualité et de la Répression des Fraudes de Ghardaïa (CACQE), BP. 179 El Atteuf, 47120 Ghardaïa, Algeria.
[3]Laboratoire d'Analyses Médicales " Ibn Rochd ", 3 rue Khemisti, Ghardaïa 47000, Algeria.
[4]Laboratoire d'Analyses médicales " El Amel", Avenue Si El Haouès, Cité Bahmid, 30000 Ouargla, Algeria.
[5]Département d'Agronomie. Laboratoire Bio-Ressources Sahariennes, Préservation et Valorisation, Faculté des Sciences de la Nature et de la Vie. Université Kasdi Merbah, Avenue 1er novembre, 30000 Ouargla, Algeria.
[6]Faculté des Hydrocarbures des Energies Renouvelables des Sciences de la Terre et de l'Univers, Université Kasdi Merbah, 30000 Ouargla, Algeria.
[7]Field Crop Development Centre, Alberta Agriculture and Forestry, 5030 – 50 Street, Lacombe, Alberta T4L1W8, Canada.
[8]Laboratoire de Biologie des Systèmes Microbiens (LBSM), Ecole Normale Supérieure de Kouba, Alger, Algeria

Abstract. In this study, we evaluated the influence of temperature on the antibacterial activity against *Escherichia coli* HO25 by measuring the inhibition zone diameters on a agar diffusion method. The antibiotic sensitivity profile was determined by testing four values of temperatures: 25, 30, 37 and 44 °C. The results obtained, indicated that there is no significant difference between 30 and 37 °C (Tukey test). Consequently, the temperature 37 °C could be replaced by 30 °C in medical and research laboratories for agar diffusion method. Furthermore, we observed that except gentamicin, all antibiotics used showed no change in resistance profile by using different temperatures.

***Keywords*:** *temperature*, *antibiotic*, *Escherichia coli HO25*, *antibiogram*, *agar diffusion method*

1. Introduction

Many studies showed that some patients do not respond well to antibiotherapy due to the increasing of bacterial resistance. This serious problem makes necessary to found more therapeutic strategies such as testing new antibacterial molecules.

In vitro testing can help to determine the activities of new antibacterial drugs (antibiotics) and to find the accurate therapy. Many methods have been reported for antibacterial and antifungal susceptibility testing of medical, veterinary, industrial and/or environmental bacterial strains [1-3]. Generally, agar-based methods are easy and quick and could be good choices [4]. Among the widely used techniques, the agar diffusion (disk diffusion) method is most used in medical and research laboratories, because of its high degree of reliability towards the standardization of the antibiotic concentration and its relative ease of use [5].

* Corresponding author.
E-mail: **noureddine_bouras@yahoo.fr** (Said A.).
Address: Université de Ghardaïa, BP 455, Ghardaïa 47000, Algeria

Moreover, the disk diffusion method was recommended by the Algerian Network for Monitoring the Resistance of Bacteria to Antibiotics [6].

To our knowledge, very little data has been published on the effect of temperature in disk diffusion susceptibility testing. The goal of this study is to evaluate the effect of temperature on the inhibition zone diameters of *Escherichia coli* (HO25) by using the disk diffusion technique.

2.- Materials and methods

2.1. Bacterial strain

The bacterial strain *Escherichia coli* (HO25) [*Enterobacteriaceae*, *Enterobacteriales*, *Gammaproteobacteria*, *Proteobacteria*] was provided from the hospital of Ouargl (Algeria). Bacterial identification was verified by chromogenic medium (chromagar).

2.2. Culture medium and antibiotics

The antibiotic sensitivity profile was determined by using Mueller-Hinton agar (MHA) purchased from Imen Laboratory. Four temperatures have been used for incubation: 25, 30, 37 and 44 °C.

A total of eight antibiotic disks (supplied by Himidia) were used in this study: AMP: Ampicillin (10 µg); AMC (30 µg): Amoxicillin (20 µg) + calvulanic acid (10 µg); CEP: Cephalothin (= cephalotin = cefalotin) (30 µg); CX: Cefoxitin (30 µg); CTX: Cefotaxime (30 µg); GEN: Gentamicin (10 µg); FOS: Fosfomycin (200 µg); and COT (25 µg): Co-Trimoxazole: Trimethoprim (1.25 µg)/sulfamethoxazole (23.75 µg). Inhibition zones were measured using a ruler. The antibacterial activity was estimated by measuring the diameter of the inhibitory zone. Each value is the average of three trials.

2.3. Agar overlay disk diffusion testing

Susceptibility testing was based on the agar disk diffusion method. From fresh grown culture of *E. coli* HO25 (20 h), a few number of colonies were suspended in 10 mL of sterilized saline water. The inoculum density of the liquid cultures was adjusted to a concentration of 0.5 McFarland.

Petri dishes (90 mm in diameter) containing 20 mL of each medium were allowed to solidify at room temperature. A cotton swab was used for inoculation. Antibiotic disks were placed onto the overlay Petri dishes and all plates were incubated, for 24 h, at different temperatures: 25, 30, 37 and 44 °C.

2.4. Statistical analysis

Data collected were statistically analyzed by factorial ANOVA and Tukey test, using XLSTAT 2014 at the probability level $P < 0.05$. The correlation test (for temperature) is carried using STATISTICA 10.

3. Results and discussion

The obtained results indicated that there is a significant effect of temperature, and also a significant effect of different antibiotics tested ($P < 0.05$). Furthermore, a significant effect of interaction "temperature × antibiotics" was also observed ($p < 0.05$). The effect of different temperatures used are summarized in Table 1, and also presented in Figure 1.

Table 1. Inhibition zone diameters (in mm) obtained for each temperature/antibiotic combination of *Escherichia coli* HO25.

T (°C)	AMP	AMC	CEP	CX	CTX	GEN	FOS	COT
25	0	0	0	0	18.5±0.5 ^{c}	19.0±0.0 ^{c}	37.0±0.0 ^{b}	0
30	0	0	0	0	14.5±0.5 ^{e}	12.5±0.5 ^{f}	37.16±0.3 ^{b}	0
37	0	0	0	0	12.5±0.5 ^{f}	11.5±0.5 ^{fg}	38.5±0.5 ^{b}	0
44	0	0	0	10±2.0 ^{g}	18.5±0.5 ^{c}	16.5±1.5 ^{d}	43.0±1.0 ^{a}	0

Data are expressed as means ± SD and the most significant groups for each treatment are mentioned according to Tukey test. Values shown are the average mean values ± standard deviation.

AMP: Ampicillin (10 µg); AMC: Amoxicillin + calvulanic acid (30 µg); CEP : Cephalothin (30 µg); CX : Cefoxitin (30 µg); CTX : Cefotaxime (30 µg); GEN: Gentamicin (10 µg); FOS: Fosfomycin (200 µg); COT: Co-Trimoxazole: Trimethoprim/sulfamethoxazole (25 µg).

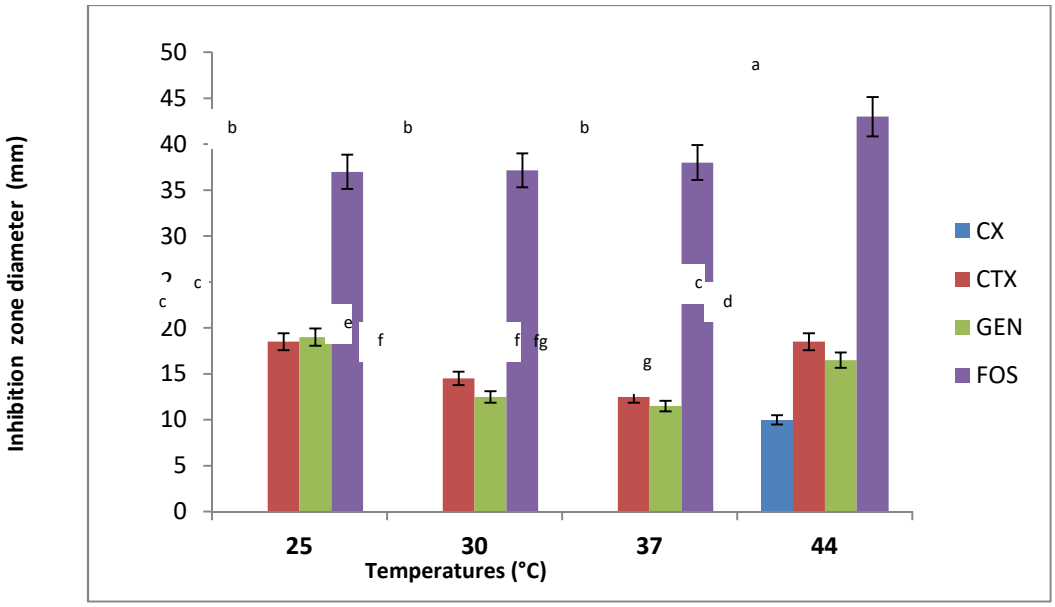

Fig. 1. Inhibition zone diameters (in mm) of *Escherichia coli* HO25 obtained for each temperature tested with four antibiotics [CX : Cefoxitin (30 µg), CTX : Cefotaxime (30 µg), GEN: Gentamicin (10 µg) and FOS: Fosfomycin (200 µg)].

The statistical analyses showed on different temperatures tested (25, 30, 37 and 44 °C) that *Escherichia coli* HO25 is very sensitive to FOS (inhibition zone ranging between 37 and 43 mm); moderately sensitive to CTX (inhibition zone ranging between 12.5 and 18.5 mm) and GEN (inhibition zone ranging between 11.5 and 19.0 mm); very resistant to CX (inhibition zone ranging between 0 and 10 mm); and totally resistant to CEP, AMC, AMP and COT.

Based on statistical analyses we noticed that there is no significant difference between 30 and 37 °C (Tukey test). Consequently the 37 °C could be replaced by 30 °C to produce antibiograms in medical and research laboratories. However, the values of temperatures 25 and 44 °C showed the most significant effect compared to other used temperatures (30 and 37 °C).

Table 2 indicated the zone diameter breakpoints of antibiotics used for *Enterobacteriaceae* (including *E. coli*).

Table 2. Performance standards for antimicrobial susceptibility testing used for *Enterobacteriaceae* [5].

	R	I	S
AMP	≤ 13	14-16	≥ 17
AMC	≤ 13	14-17	≥ 18
CEP	≤ 14	15-17	≥ 18
CX	≤ 14	15-17	≥ 18
CTX	≤ 22	23-25	≥ 26
GEN	≤ 12	13-14	≥ 15
FOS	≤ 12	13-15	≥ 16
COT	≤ 10	11-15	≥ 16

R = Resistant, S = Susceptible, I = Intermediate.

Table 3 showed the results of the effect of different temperatures on the inhibition zones of different antibiotic tested against *E. coli* HO25.

Table 3. Antibiotic susceptibility pattern of *Escherichia coli* HO25 (R = Resistant, S = Susceptible, I = Intermediate).

T (°C)	AMP	AMC	CEP	CX	CTX	GEN	FOS	COT
25	R	R	R	R	R	S	S	R
30	R	R	R	R	R	R/I	S	R
37	R	R	R	R	R	R	S	R
44	R	R	R	R	R	S	S	R

AMP: Ampicillin (10 µg); AMC: Amoxicillin + calvulanic acid (30 µg); CEP : Cephalothin (30 µg); CX : Cefoxitin (30 µg); CTX : Cefotaxime (30 µg); GEN: Gentamicin (10 µg); FOS: Fosfomycin (200 µg); COT: Co-Trimoxazole: Trimethoprim/sulfamethoxazole (25 µg).

Based on Table 3, we observed that, except GEN (at 30 °C), all antibiotics used showed no change in resistance profile by using different temperatures. This finding confirmed that the incubation temperature 37 °C could be replaced by 37 °C. Furthermore, the results indicated that in some critical conditions, such as ichthyopathology, other values of temperatures ranging between 25 and 44 °C could also be used for antibiograms.

The Figure 2 showed the theoretical correlation test of inhibition zone diameters for temperatures ranging between 0 and 65 °C for three active antibiotics: cefotaxime, gentamicin and fosfomycin. The results indicated that almost no change in inhibition zone diameters for cefotaxime and gentamicin for all used temperatures. In the case of fosfomycin, the inhibition zone diameters ranged from 28.5 mm (at 0 °C) to 48.2 mm (at 65 °C), have no effect on resistance profile (always resistant). These results confirmed that a wide range of temperatures could also be used for antibiograms, especially in some special cases such as ichthyopathology and other antibiograms applied in different diseases of aquatic animals.

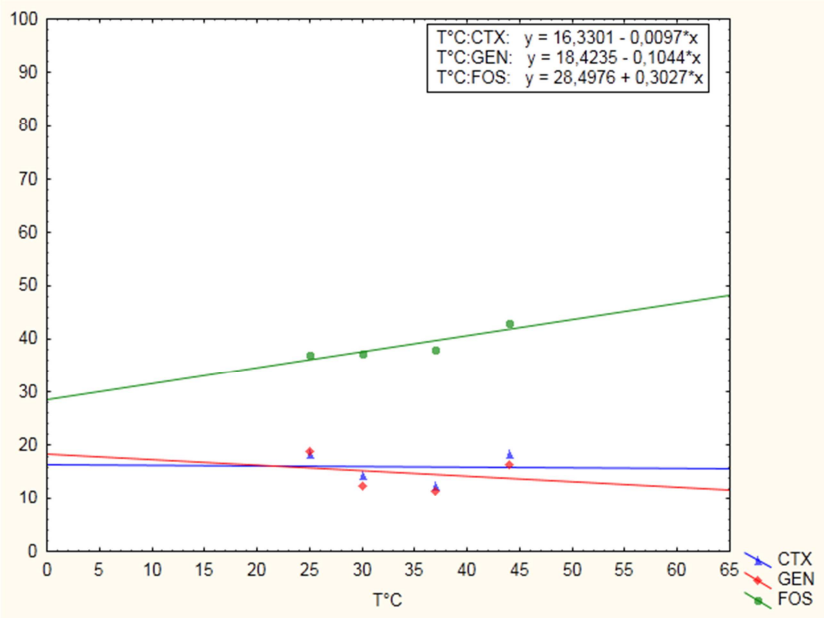

Fig. 2. The theoretical correlation test of inhibition zone diameters (in mm) of three antibiotics against *E. coli* HO25 for temperatures ranging from to 0 to 65 °C.

CTX : Cefotaxime (30 µg), GEN: Gentamicin (10 µg) and FOS: Fosfomycin (200 µg).

Michel and Bassalert [7], reported that the influence of the temperature in the laws of the diffusion is a classic data in physics, and it was predictable that this phenomenon is manifested in the case of the antibiogram. The same authors reported that the diameters significantly differed according to the

incubation temperatures between 22 and 37 °C. This variation between 22 and 37 °C could reach 7 to 8 mm [7]. However, the resistance profile did not changed for all bacterial strains with all antibiotic tested [7]. *In vitro* testing can help to determine the activities of new drugs and to find the right therapy. Agar-based methods are quick and easy and could be good options [8].

it's necessary to mention that for all tested temperatures, the reported data have been obtained by using the same culture medium (Mueller-Hinton agar) and under the same conditions of pH (7.00); however, only one strain of *E. coli* has been used in this study (HO25), and strain should be taken into consideration for any final conclusion.

Although the reference methods recommend the use of 37 °C as incubation temperature for agar testing, it seems that other values of temperatures ranging between 25 and 44 °C could also be used to obtain the same results (without any change in resistance profile).

4. Acknowledgements

The authors would like to thank those who have contributed in this study: the Director of CACQE, El Atteuf (Ghardaia): M. Rezzag Moussa and the researchers of CACQE: Moulay Abdallah Karima, Kchaou Nawel, Ouled Belkhir Mohamed and Hachemi Taher. The Director of the "Laboratoire d'Analyses Médicales Ibn Rochd" : Dr. Ammi Saïd Mustapha. The Director and the researcher of the "Laboratoire d'Analyses Médicales El Amel", respectively : Dr. Hadjadj Sofiane and M. El-Alouani Salah. In addition, the authors would like to thank Dr. Kheireddine Aliouche for providing strain *E. coli* HO25.

5. References

[1] Perreten V, Kollöffel B, Teuber M. (1997) Conjugal transfer of the Tn916-like transposon TnFO1 from *Enterococcus faecalis* isolated from cheese to other Gram-positive bacteria. Syst Appl Microbiol;20:27-38.

[2] Perreten V, Giampa N, Schuler-Schmid U, Teuber M. (1998) Antibiotic resistance genes in coagulase-negative Staphylococci isolated from food. Syst Appl Microbiol;21:113-120.

[3] Butaye P, Van Damme K, Devrieze LA, Van Damme L, Baele M, Lauwers S, Haesebrouck F. (2000) *In vitro* susceptibility of *Enterococcus faecium* isolated from food to growth-promoting and therapeutic antibiotics. Int J Food Microbiol;54:181-187.

[4] Matar MJ, Ostrosky-Zeichner L, Paetznick VL, Rodriguez JR, Chen E, Rex JH. (2003) Correlation between E-Test, disk diffusion, and microdilution methods for antifungal susceptibility testing of fluconazole and voriconazole. Antimicrob Agents Chemother;47:1647–1651.

[5] Huys G, D'haene K, Swings J. (2002) Influence of the culture medium on antibiotic susceptibility testing of food-associated lactic acid bacteria with the agar overlay disc diffusion method. Lett App Microbiol;34:402-406.

[6] Standardisation de l'antibiogramme à l'échelle nationale (Médecine humaine et vétérinaire). République algérienne démocratique et populaire. Réseau Algérien de la Surveillance de la Résistance des Bactéries aux Antibiotiques. Document édité avec la collaboration de l'OMS, 6ème édition, 2011.

[7] Michel C, Bassalert JF. (1982) Influence de la température sur les résultats de l'antibiogramme pratiqué par la méthode de diffusion en ichthyopathologie. Ann Rech Vet, INRA Editions;13:245-250.

[8] Fernández-Torres B, Carrillo-Muňoz A, Inza I, Osep Guarro J. (2006) Effect of culture medium on the disk diffusion method for determining antifungal susceptibilities of dermatophytes. Antimicrob Agents Chemother;50:2222-2224.