# Exploring β -Lactamases genes and Biofilm formation in *E. coli* isolated from Wastewater, Morocco.

**AIDDI Amine[1,2]*; KHAZAZ Aboubakr[2,3]; MGUILD Hafsa[2,4]; BENZAARATE Ihssane[2, 3]; BOURJILAT Fatna[2]; ZERDANI Ilham[1]; NAYME Kaotar[2].**

[1]Microbiology Unit, Laboratory of Ecology and Environment, Department of Biology, Faculty of Sciences Ben M'sik, Hassan II University, Casablanca, Morocco.[2]Molecular Bacteriology Laboratory, Centre de sérums et vaccins (Institut Pasteur du Maroc), Casablanca ; Morocco. [3]Microbiology and Antimicrobial Agents research team, LB2VE, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida; Morocco. [4]Research Laboratory of Microbiology, Infectious diseases and Microbial Valorization, Mohammed VI University of Sciences and Health (UM6SS), Casablanca, Morocco·

***Corresponding author address:** aiddiamine@gmail.com*

## Introduction

The rise of antibiotic resistance poses a global public health challenge. Wastewater environments, often overlooked, serve as reservoirs for antibiotic-resistant bacteria, fostering the dissemination of resistance genes and posing health risks through various exposure routes. Our study, conducted in Morocco, focuses on the phenotypic and genotypic characterization of antibiotic resistance and biofilm formation in ***Escherichia coli* (*E. coli*)** strains isolated from wastewater samples.

## Purpose of the study

This study underscores the pivotal role of wastewater in the perpetuation and dissemination of antibiotic resistance, emphasizing the necessity for nuanced wastewater management strategies to curb resistance propagation and safeguard public health.

**Fig. 1: Sampling sites**

## Methods

- **Sampling sites**: Over a period of two months, from the 1st February to 31st March 2023, wastewater samples were systematically collected from two distinct sites, **Anza** (Latitude: 30.45431; Longitude: -9.65362) and **Oued Lahouar** (Latitude: 30.40016; Longitude: -9.60008), situated in the Agadir city, Morocco. Most of the sampling sites were impacted by pollution from agricultural, industrial, and domestic origins (**Fig. 1**).
- **Antimicrobial Susceptibility Testing** was performed by the diffusion method on gelose MH (BioRad), according to the recommendations of CASFM/EUCAST 2022.
- **Isolation and identification of bacterial strains:** Isolation of *E. coli* strains (**n=14**) involved the use of a chromogenic medium Brilliance UTI Clarity Agar (Oxoid). Identification was achieved through biochemical and 16S rRNA methods.
- **Biofilm formation on polystyrene microtiter plates:** *E. coli* isolates were incubated on the microplate at various time points ranging from 2 to 24 hours, the absorbance at 620 nm was measured using a microplate reader (800 TS Microplate Reader).
- **Detection of β-lactamases genes:** PCR assays were conducted to identify genes encoding resistance to β-lactams (***bla*ctx-M, *bla*TEM, *bla*SHV**).

## Results

- A high prevalence of resistance to various antimicrobial agent was noted (**Fig. 2**).

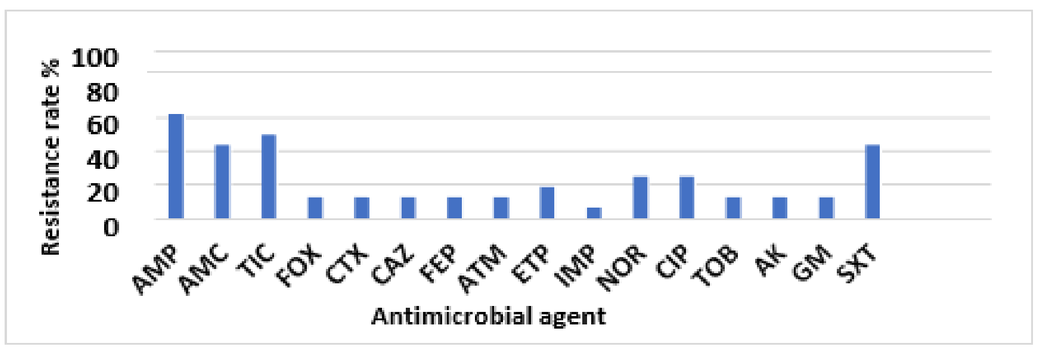


**Fig. 2: Antibiotic resistance rate of *E.coli* isolated from Wastewater.**

**AMP: Ampicillin; AMC: Amoxicillin/clavulanate; TIC: Ticarcillin; FOX: Cefoxitin; CTX: Cefotaxime; CAZ: Ceftazidime; FEP: Cefepime; ETP: Ertapenem, IMP: Imipenem; NOR: Norfloxacin; CIP: Ciprofloxacin; TOB: Tobramycin; AK: Amikacin; GM: Gentamicin; SXT: co-tromoxazole.**

- Genetic analysis revealed the presence of ***blactx-M*1** in one isolate (**Table 1**).

**Table 1: β-lactamases genes detected in *E.coli* isolated from Wastewater.**

| Code | Isolate | City | β-lactamases genes | Antibiotic resistance profile |
|---|---|---|---|---|
| Ec A8 | E.coli | Agadir | blactx-M1 | AMP, AMC, TIC, CTX, CAZ, FEP, ATM |

- Assessment of biofilm formation on polystyrene microtiter plates showed that the most strains begin biofilm formation between 4h to 18h of incubation, with a moderate formation (20%) after 4h and 22h (**Fig. 3**).

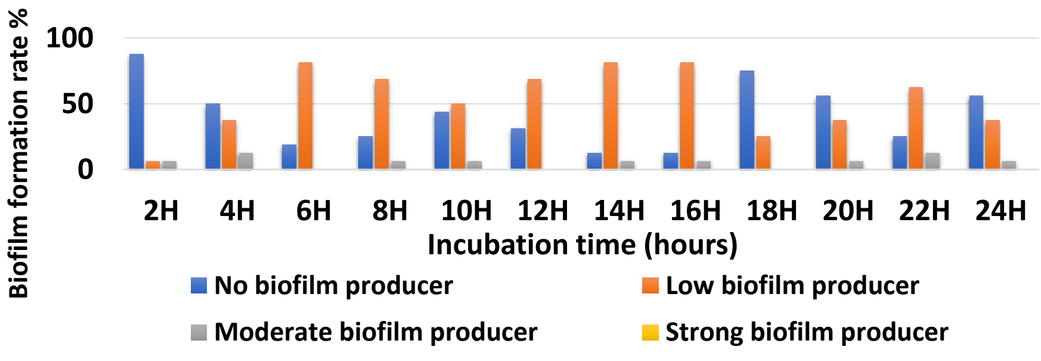


**Fig. 3: Biofilm formation Kinetics of *E.coli* strains isolated from Wastewater,**

## Conclusion

Our findings highlight the critical role of wastewater as a reservoir for antibiotic resistant bacteria emphasizing the need for tailored wastewater management strategies to mitigate resistance dissemination and protect public health.