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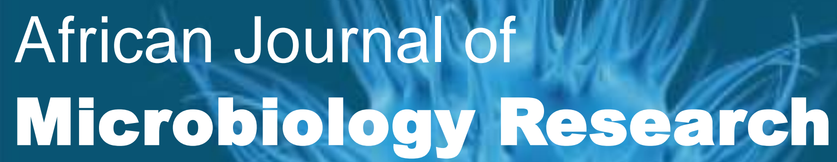

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# Table of Content

**Antibacterial activity of endophytic fungus, Penicillium griseofulvum MPR1 isolated from medicinal plant, Mentha pulegium L.**
Amina ZERROUG, Nouari SADRATI, Rasime DEMIREL,
Sabrina BAKLI and Daoud HARZALLAH

**Health and nutritional quality assessment of salmonella-contaminated poultry products in sub-Saharan Africa; A case of cote d'ivoire**
KOFFI Ahua René, KOUADIO-N'GBESSO Nadège, OUASSA Timothée,
ATOBLA Koua, KOUAME N'Zébo Désiré and DADIE Adjéhi

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African Journal of Microbiology Research

*Full Length Research Paper*

# Antibacterial activity of endophytic fungus, *Penicillium griseofulvum* MPR1 isolated from medicinal plant, *Mentha pulegium* L.

**Amina ZERROUG[1]*, Nouari SADRATI[1], Rasime DEMIREL[2], Sabrina BAKLI[3] and Daoud HARZALLAH[3]**

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**In the present study, an endophytic fungus with a considerable antimicrobial activity was isolated from the medicinal plant *Mentha Pulegium* L. The morphological and molecular identification revealed that this fungus is a *Penicillium griseofulvum*. A preliminary screening was done to choose the suitable culture medium for a maximum production of the bioactive compounds using the dual-culture agar diffusion assay. The malt extract agar (MEA) and potato dextrose agar (PDA) media were the suitable, and the MEA was selected for the further study. Extraction was done with three solvents, n-Hexane, dichloromethane and ethyl acetate, and the crude extracts were tested against Gram-positive and negative bacteria. A high activity was found with ethyl acetate and dichloromethane crude extracts against all bacteria with a maximal inhibition zones of 45.5 and 41 mm respectively obtained against *Escherichia coli*. The minimum inhibitory and bactericidal concentrations (MIC, MBC) of ethyl acetate crude extract were evaluated using the broth micro-dilution method. A MIC of 50 µg / ml on Gram-negative bacteria and of 100 µg / ml on Gram-positive bacteria was found. The $MBC_S$ ranged from 50 and 200 µg/ml. The time kill study has revealed a bactericidal activity of the *Penicillium griseofulvum* crude extract. At 24 h and for all concentrations (MIC× 2 and MIC× 4), 100% killing of the bacterial cells was achieved. These results prove that the extract of *Penicillium griseofulvum* can be a promising source of important bioactive molecules.**

**Keys words:** Antibacterial activity, endophytic fungi, *Mentha pulegium* L., *Penicillium griseofulvum.*

## INTRODUCTION

Medicinal plants like all plants interact continously with different microorganisms, like fungi (both pathogenic and beneficial), living on the surface of plants (epiphytes) and their roots (mycorhizes) and even inside (endophytes)

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(Passari et al., 2015). Endophytic fungi live asymptomatically in the intercellular and/or intracellular spaces of healthy plants for at least a part of their life (Dos Banhos et al., 2014). All parts (leaves, stems, roots, barks, etc) of all plants examined till date are colonized by endophytic fungi (Shen et al., 2014). The relationships between the endophytic fungi and their hosts can change between neutral association to mutualistic, pathogenic or saprophytic interactions depending on genetic dispositions of the two partners, environmental factors and time (Martín et al., 2013).

The increasing resistance of pathogenic micro-organisms to antibiotics in the world, and recently in Algeria, leading to the resurgence of more complex infections (Baba Ahmed-Kazi Tani and Arlet, 2014), has pushed researchers to discover new bioactive molecules. The capacity of endophytic fungi to biosynthesize bioactive secondary metabolites (more than 20.000 bioactive molecules) (Marson Ascêncio et al., 2014) and their various biological activities such as antimicrobial, anti-viral, anticancer, antioxidant, antidiabetic, and anti-inflammatory activity (Sadrati et al., 2013; Bashyal et al., 2014; Shah et al., 2015; Jouda et al., 2016a; Pan et al., 2016; Chen et al., 2016; Singh and Kaur 2016; Yao et al., 2017) have made this fungus to be important, as an important source of new bioactive secondary metabolites potentially useful for human medicine and other domains.

Medicinal plants have been well studied because they harbor rare microorganisms, that imitate the chemistry of their hosts, and making the same molecules or derivate more active than those synthesized by their hosts. It is also probable that the bioactivity of medicinal plants may be due to endophytes living inside plants by genetic exchange (Tayung et al., 2011).

*Mentha pulegium* L., commonly known as pennyroyal, belongs to the Lamiaceae family. It is native to America, Europe, North Africa and in Asia Minor and Near East (Mahboubi and Haghi, 2008). It is widely used in traditional medicine (food poisoning, bronchitis, diuretic, flatulence, intestinal colic), food like culinary herb, cosmetics and aromatherapy (Hajlaoui et al., 2009; Aires et al., 2016). Only Debbab et al. (2009) and Teiten et al. (2013) have studied the endophytic fungi of this plant, and have determined the cytotoxicity and anticancer activity of the bioactive metabolites of the endophytic fungus, *Stemphylium globuliferum*. However, the antibacterial activity of endophytic fungus of this plant has not been examined; for this reason we selected this plant for isolation of this endophytic fungus in order to investigate its antibacterial activity.

## MATERIALS AND METHODS

### Sample collection and fungal isolation

Endophytic fungi were isolated from the healthy medicinal plant *Mentha pulegium* L., collected from the mountain of Megriss Setif Algeria in June 2014. They were identified by faculty botanists, according the following method. Different tissues (leaves, stems and roots) were washed with tap water, cut into small pieces (leaf pieces size of approximately 5.0 × 5.0 cm; stems and root pieces of approximately 5.0 cm long); they were surface-sterilized by sequential immersions in 70% (v/v) ethanol for 1 min, 3% sodium hypochlorite for 4 min and 70% (v/v) ethanol for 1 min and then rinsed thrice in sterilized distilled water for 1 min each (Petrini, 1986; Pimentel et al., 2006; Xie et al., 2016). After drying on sterile filter paper, each segment was cut into 0.5 cm and aseptically transferred onto Petri-dishes containing Potato Dextrose Agar (PDA) (Sigma-Aldrich; pH 5.6 ± 0.2) supplemented with 100 mg/l of Gentamicine to suppress bacterial growth. The dishes were incubated at 28°C and checked continuously for the growth of endophytic fungi colonies. Each fungus emerging from the segments was transferred to fresh PDA plates and PDA slants for conservation at 4°C.

### Initial screening of antibacterial activity

#### *Tested bacteria*

The endophytic fungi were screened for their antibacterial activity using the pathogenic bacteria, *Bacillus cereus* ATCC 10876, *S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922. Each bacterium was grown in nutrient agar (NA) at 37 °C for 24 h, and diluted until the concentration got to $10^8$ c.f.u. / ml by spectrophotometry (OD $_=$ 0.08-0.1 at 625 nm) (Powthong et al., 2013).

#### *Preliminary antibacterial assay*

Antibacterial activity of the endophytic fungi was performed using the method of agar plug diffusion explained by Powthong et al. (2013) and Sahani et al. (2017), with some modification. Briefly, pure cultures of the endophytic fungi were cultivated on the surface of PDA at 28°C for 14 days. Then, a small disk of fungal colony was cut (6 mm diameter) using a sterile cork borer and placed on previously inoculated Muller Hinton Agar (MHA) (Sigma- Aldrich; 7.3±0.2) plates. A disk of PDA without fungi was used as negative control. The Petri dishes were refrigerated at 4°C for 4 h for complete diffusion of antibacterial compounds from the fungal disks, then these plates were incubated at 37°C for 24 h. Antibacterial activity was determined by measuring zones of inhibition produced by the endophytic fungi. The endophytic fungus MPR1 displaying good antibacterial activity was selected for further study.

### Identification

The fungus was initially identified based on morphological characteristics using the standard mycological manuals and microscopic examination (Rajeswari et al., 2017). For molecular identification, isolate was grown on PDA plate for 7 days at 25°C. DNA was extracted from culture using the Ultraclean Microbial DNA Isolation Kit (MoBio, Solana Beach, CA, USA) following the manufacturer's protocols.

Molecular characterization of the isolates was performed using sequencing of the internal transcribed spacer (ITS) region that is standard gene regions. For this aim, ITS gene regions were amplified with the primers V9G, 5'-TTACGTCCCTGCCCTTTGTA-3' (forward) and LS266, 5'-GCATTCCCAAACAACTCGACTC-3' (revers) (Chen et al., 2016; Kadaifciler and Demirel, 2017) by polymerase chain reaction [Veriti® 96-Well ThermalCycler (Applied

Biosystems®)].

Amplification was carried out with a total volume of 25 µl containing 1 µl of genomic DNA, 2.5 µl of 2.5 µM forward and reverse primers, 2.5 µl of 10 × Taq buffer + KCl-$MgCl_2$ (Bioline, UK), 2.5 µl of 25 µM $MgCl_2$ (Fermentas, CA, USA), 2µl of 2.5 µM dNTP mix, 0.25 µl of 5 U / µl Taq DNA polymerase (Bioline, UK), and 11.75 µl of nuclease free water under the following condition: Initial denaturation step for 5 min at 95°C, followed by 35 cycles of denaturation for 45 s at 95°C, annealing for 30 s at 56 °C and extension of 2 min at 72°C, followed by a final extension at 72°C for 6 min (Kadaifciler and Demirel, 2017). PCR product was separated by agarose gel electrophoresis (1% W/v in 1xTAE) and visualized by GelRed staining. PCR products were purified using EXOSAP-IT (Affimetrix) and sequenced using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') with a CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA) following the method of Demirel et al. (2013).

**Data analyses**

The sequence was compared with those deposited in the NCBI GenBank Database via BLAST searches (Altşchul et al., 1990), and aligned using the Muscle in MEGA7.0 software package together with the other sequences of morphologically and phylogenetically related type species that were obtained from NCBI GenBank (Kumar et al., 2016). The aligned data sets were analyzed using Maximum Likelihood analysis based on the Tamura Nei model (Tamura and Nei, 1993) as implemented in the MEGA 7.0 with 1000 bootstrap replications. Gaps and missing data in all the position were eliminated. *Penicillium crustosum* (AF033472) was used as out group. The obtained sequence data were deposited in NCBI GenBank, and an accession number was obtained.

**Selection of suitable culture media**

To select the best medium in which the isolate exhibited maximum inhibition zone, the maximum antibiotic production was then used for further study; MPR1 was cultivated on the surface of different culture media, potato dextrose agar (PDA), Sabouraud's dextrose agar (SDA) (dextrose, 40 g/l; peptone, 10 g/l; Agar, 15 g/l; pH 5.6 ± 0.2), yeast extract agar (YEA) (yeast extract, 3 g/l; Peptone, 5 g/l; Agar, 15 g/l; pH 7.2 ± 0.2), malt extract agar (MEA) (malt extract, 20 g/l; dextrose, 20 g/l; peptone, 6 g/l; agar, 15 g/l; pH 5.4 ± 0.2), yeast malt extract agar (YMEA) (yeast extract, 3 g/l; Malt extract, 3 g/l; dextrose, 10 g/l; peptone, 5 g/l; agar, 15 g/l; pH 6.2 ± 0.2) at 28°C for 14 days. The agar plug diffusion method was done as described above against four Gram-positive bacteria *B. cereus* ATCC 10876, *E. faecalis* ATCC 49452, *S. aureus* ATCC 25923, Methicillin-resistant *S. aureus* ATCC 43300 and four Gram-negative bacteria *Citrobacter freundii* ATCC 8090, *Salmonella typhimurium* ATCC 13311, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922.

**Fermentation and extraction**

The endophytic fungus was cultivated on Malt Extract Broth (MEB) (Malt extract, 20 g/l; Dextrose, 20 g/l; Peptone, 6 g/l; pH 5.4 ± 0.2) by inoculating agar blocks of actively growing pure culture (6 mm in diameter) in 250 ml Erlenmeyer flasks containing 100 ml of the medium MEB for 21 days at 28 ± 2°C and 150 rev / min. Fungal mycelia were separated using Whatman filter paper and further centrifuged at 4000 rev / min / min for 5 min (Mai et al., 2013; Astuti et al., 2014).

In order to choose the best extraction solvent, three organic solvents were used as n-Hexane, dichloromethane and ethyl acetate. The extraction method was performed based on the method explained by Saraswaty et al. (2013). Briefly, the supernatant of liquid fermentation was extracted with an equal volume of organic solvent starting with the non-polar organic solvent (n-Hexane). The solution was then mixed for 10 min and allowed to stand. The layer of n-Hexane was then collected and evaporated under vacuum using a rotary evaporator (BÜCHI). The remaining aqueous phase was re-extracted by the other solvents using the same method. The crude extracts were then dissolved in dimethyl sulphoxide (DMSO) and kept at 4°C.

**Evaluation of antibacterial activity of crude extracts**

Antibacterial activity of secondary metabolites extracted from MPR1 was done following an agar well diffusion method described by Fatima et al. (2016) against *B. cereus* ATCC 10876, *S. aureus* ATCC 25923, Methicillin-resistant *S. aureus* ATCC 43300, *C. freundii* ATCC 8090, *S. typhimurium* ATCC 13311 and *E. coli* ATCC 25922.

100 µl ($10^8$ c.f.u. / ml) of bacterial inoculums was spread on MHA plates; then wells (6 mm diameter) were made and 25 µl of each extract was poured into these wells. DMSO was used as negative control. After incubation of 24 h at 37°C, the diameter of each zone of clearance on plates was measured and used as an indicator of antibacterial activity of the extract.

**Determination of minimum inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs)**

The minimum inhibitory concentrations (MICs) and the minimal bactericidal concentrations (MBCs) were determined using broth micro-dilution method following the protocol established by CLSI (2012). The ethyl acetate extract was diluted in DMSO to have an initial concentration of 1600 µg / ml and 2-fold serial dilution was done (1600, 800, 400, 200, 100, 50, 25, 12.5, 6.25, 3.125 µg / ml), 90 µl of sterile Mueller Hinton Broth (MHB) (Sigma-Aldrich; pH 7.4 ± 0.2); the wells were then inoculated with standard size of bacterial suspension of 5× $10^6$ c.f.u. / ml. The DMSO and the standard drugs (Imipenem and Gentamycin) were used as negative and positive control, respectively.

After 24 h of incubation at 37°C, 20 µl of 0.5% of 2, 3-5 Triphenyltetrazolium chloride aqueous solution was added to each well. After a second incubation for 30 min at 37°C, the lowest concentration of ethyl acetate crude extract which did not show any visual color change was recorded as the MIC. All wells that showed growth inhibition were subcultured on MHA. After incubation of 24 h at 37°C, the lowest concentration that exhibited no visible growth was considered to be the MBC.

**Time kill study**

To determine the bactericidal or bacteriostatic effect of *Penicillium griseofulvum* ethyl acetate extract, the time kill test was done against Methicillin-resistant *S. aureus* ATCC 43300. Into three tubes of MHB medium with different concentrations of extract (MIC, MIC× 2 and MIC× 4), the bacterial suspension was transferred to obtain an initial inoculum cell density of approx. 5 × $10^5$ c.f.u. / ml; a fourth tube was used as the growth control containing the MHB and the bacterial suspension. These four tubes were then incubated at 37°C in a rotary shaker at 150 rev / min for varied time intervals (0, 1, 2, 4, 6, 12 and 24 h). For viable cells counts, in each time interval, a 0.1 ml sample was removed, diluted and spread onto

**Table 1.** Preliminary antibacterial activity of endophytic fungus (n=2, means).

| Parameter | Inhibition diameter zone (mm) | | | |
|---|---|---|---|---|
| | ***Bc*** | ***Sa*** | ***Pa*** | ***Ec*** |
| MPR1 | 33.5±0.71 | 31.5±0.71 | 16.5±2.12 | 47.5±0.71 |
| Negative control | 00±00 | 00±00 | 00±00 | 00±00 |

Ba, *Bacillus cereus* ATCC 10876; Sa, *Staphylococcus aureus* ATCC 25923; Pa, *Pseudomonas aeruginosa* ATCC 27853; Ec, *Escherichia coli* ATCC 25922.

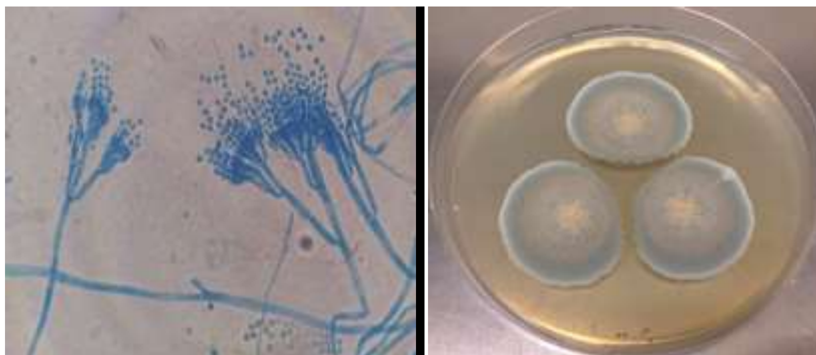

**Figure 1.** Morphological characteristics of MPR1 isolate.

MHA plates and these plates were then incubated at 37°C for 24 h and the bacterial colonies were counted.

Time kill curve (log c.f.u. / ml vs. time) was drawn for each concentration of extract and control culture, and percentages of dead cells were calculated as the following equation:

Reduction (%) $= V_0 - V_z / V_0 \times 100$

Where, V0 is the initial viable cell count and $V_z$ is the viable cell count at time z (Ibrahim et al., 2015).

Generally, the bactericidal effect is obtained with a lethality percentage of 90% for 6 h, which is equivalent to 99.99% of lethality for 24 h (Balouiri et al., 2016).

#### Statistical analysis

All experiments were performed in duplicates, and statistical analysis was carried out using SAS/STAT® 9.2 software. Group comparisons were performed using the two-way ANOVA followed by Student-Newman-Keuls multip-rang test. Results are represented as mean ± standard deviation (SD) and significant effects of treatments were determined by F values ($P \leq 0.05$).

## RESULTS AND DISCUSSION

### Isolation and preliminary antibacterial assay of endophytic fungi

The 16 endophytic fungi emerging from the surface sterilized plant segments were preliminary screened against four clinical pathogenic bacteria; this permitted a rapid and qualitative selection of the bioactive micro-organism.

According to the results of this screening, we focused our work on a single fungal isolate MPR1 that inhibited all of these pathogenic bacteria with a good activity (Table 1).

The isolate showed maximum activity against *E. coli* ATCC 25922 with an inhibition diameter zone of 47.5 mm followed by *B. cereus* ATCC 10876, *S. aureus* ATCC 25923 and *P. aeruginosa* ATCC 27853 with inhibition diameters zones of 33.5; 31.5 and 16.5 mm, respectively.

The results of this preliminary screening are in agreement with the results obtained with endophytic fungi isolated from two medicinal plants *Ocimum sanctum* and *Aloe vera* by Sahani et al. (2017), where all isolated endophytic fungi had broad-spectrum of antibacterial activity against both Gram-positive and Gram-negative pathogenic bacteria.

### Identification of endophytic fungus MPR1

The fungal isolate MPR1 was characterized by observing colony morphology, pigmentation, growth pattern and sporulation structure, and the fungus has been preliminarily identified up to genus level as *Penicillium* sp. (Figure 1).

Best-scoring maximum likelihood tree based on the Tamura–Nei model calculated using MEGA 7.0 based on ITS sequences showing the relationships of the newly

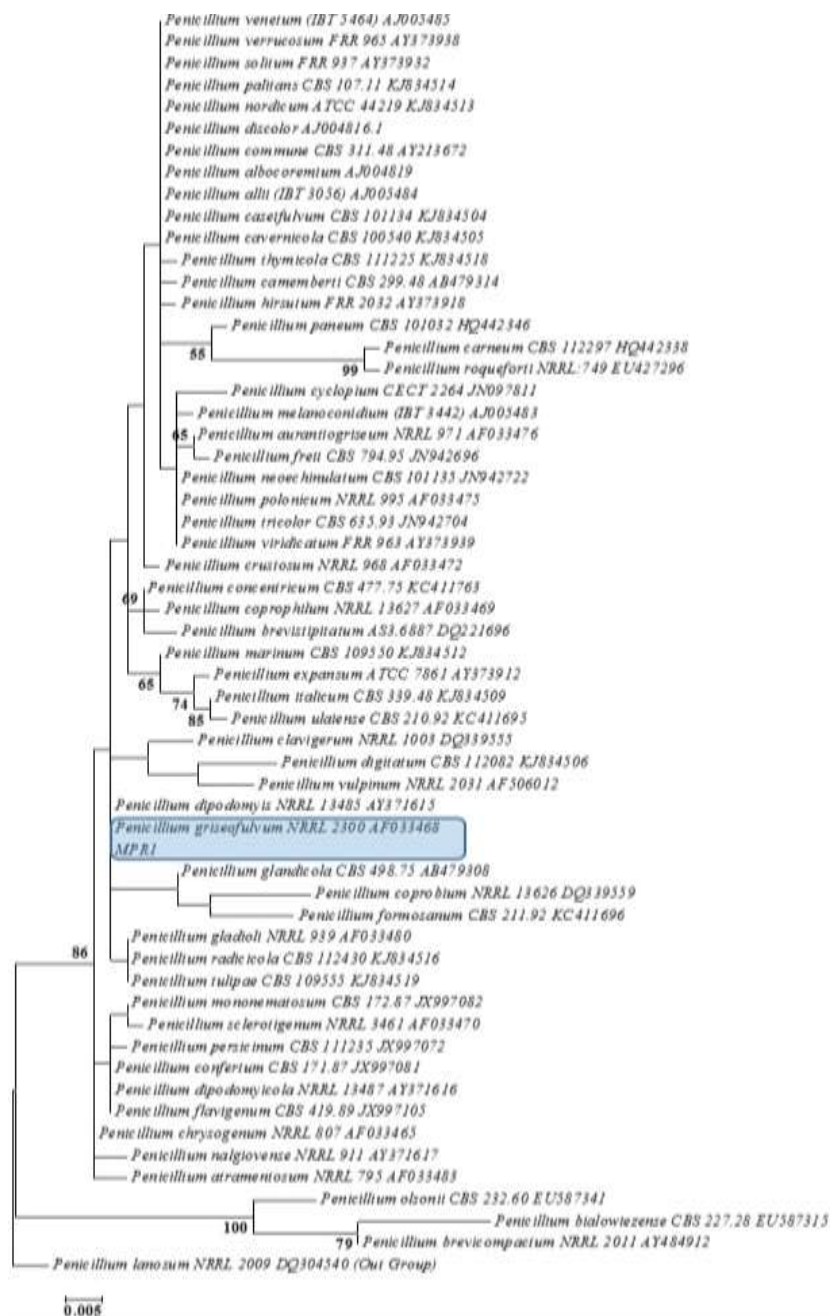


**Figure 2.** Best-scoring Maximum likelihood tree calculated using MEGA 7.0 based on ITS sequences showing the relationships of the newly generated sequence in this study with previously known taxa in NCBI GenBank.

generated sequences (GenBank accession number MH006592) in this study with previously known taxa in the NCBI GenBank. The scale bar denotes 0.002 substitutions per position. The tree with the highest log likelihood (–1444.9347) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 58 nucleotide sequences. There were a total of 451 positions in the final dataset. Figure 2 shows phylogenetic

**Table 2.** Antibacterial activity assay from *Penicillium griseofulvum* grown on five culture media (n = 2, means).

| Pathogenic bacteria | Inhibition diameter zones (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Media | | | | | | | |
| | Bc | Ef | Sa | MRSA | Cf | St | Pa | Ec |
| PDA | 32.3±1.2 | 25.3±0.6 | 39.7±0.6 | 40.3±0.6 | 33.7±2.1 | 35.0±0.0 | 15.3±1.5 | 33.0±0.0 |
| NC | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| SDA | 0.0 | 0.0±0.0 | 21.0±1.0 | 23.3±0.6 | 23.0±0.0 | 28.3±0.6 | 0.0±0.0 | 27.7±0.6 |
| NC | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| YEA | 25.0±0.0 | 10.3±0.6 | 24.3±1.5 | 26.3±0.6 | 27.0±1.0 | 25.3±0.6 | 0.0±0.0 | 23.0±0.0 |
| NC | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| MEA | 33.0±1.7 | 29.0±1.0 | 41.7±0.6 | 42.7±0.6 | 38.0±0.0 | 38.7±0.6 | 18.3±0.6 | 38.0±0.0 |
| NC | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |
| YMEA | 18.7±0.6 | 9.0±0.0 | 14.7±0.6 | 15.0±0.0 | 20.3±0.6 | 20.0±0.0 | 0.0±0.0 | 25.0±0.0 |
| NC | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 | 0.0±0.0 |

Bc, *Bacillus cereus* ATCC 10876 ; Ef, *Enterococcus faecalis* ATCC 49452; Sa, *Staphylococcus aureus* ATCC 25923; MRSA, Methiciline-resistant; *Staphylococcus aureus* ATCC 43300 ; Cf: *Citrobacter freundii* ATCC 8090, Sa, *Salmonella typhimurium* ATCC 13311; Pa, *Pseudomonas aeruginosa* ATCC 27853; Ec, *Escherichia coli* ATCC 25922; NC, Negative control.

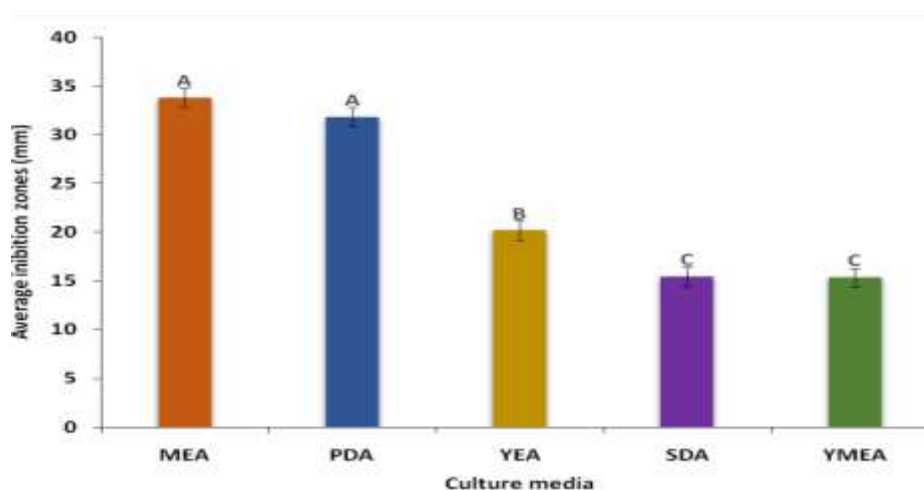


**Figure 3.** Effect of different culture media on antibacterial activity of *Penicillium griseofulvum* against all pathogenic bacteria.

tree belong to investigated taxa. The endophytic fungus MPR1 was found to be a homolog of type culture *Penicillium griseofulvum NRRL 2300* (AF033468).

The tree is rooted with *Penicillium lanosum NRRL 2009* (DQ304540) (Bootstrap 1000). The species *P. griseofulvum* has been isolated as endophyte from different host plants in different studies. For example, Conti et al. (2012) isolated *P. griseofulvum* from the leaves of *S. verticillata* (L.). Another study resulted in the isolation of *P. griseofulvum* from *Palicourea Tetraphylla* Cham. and Schltdl (Rosa et al., 2010). D'Souza and Hiremath (2015) isolated *P. griseofulvum* from seven different medicinal plants of India.

**Selection of suitable culture media**

For the optimization of the production of the endophytic fungi bioactive molecules of five different basal media PDA, SDA, YEA, MEA, YMEA were used (Table 2).

According to the statistical analysis (Figure 3), MEA and PDA (no significant differences) were found to be the best media for maximum production of antibacterial bioactive compound with average inhibition zones of 33.8 mm and 31.8 mm, respectively and the best activity was observed against Methiciline-resistant *S. aureus* ATCC 43300 (42.7 mm and 40.3 mm respectively). YEA comes in second position (20.2 mm of average inhibition zones)

**Table 3.** Antibacterial activity of different crude extracts of *Penicillium griseofulvum* (n = 2, means).

| Pathogenic bacteria | Inhibition diameter zone (mm) | | | Negative control (DMSO) |
|---|---|---|---|---|
| | Solvents | | | |
| | Ethyl acetate | Dichloromethane | n-Hexane | |
| Bc | 40.5±0.7 | 35.5±0.7 | 0.0 | 0.0±0.0 |
| Sa | 37.5±0.7 | 31.5±0.7 | 0.0 | 0.0±0.0 |
| MRSA | 41.5±0.7 | 33.0±0.0 | 0.0 | 0.0±0.0 |
| Cf | 43.0±1.4 | 36.5±0.7 | 0.0 | 0.0±0.0 |
| St | 43.5±0.7 | 36.5±0.7 | 0.0 | 0.0±0.0 |
| Ec | 45.5±0.7 | 41.0±1.4 | 0.0 | 0.0±0.0 |

Bc, *Bacillus cereus* ATCC 10876; Sa, *Staphylococcus aureus* ATCC 25923; MRSA, Methiciline-resistant *Staphylococcus aureus* ATCC 43300; Cf, *Citrobacter freundii* ATCC 8090; St, *Salmonella typhimurium* ATCC 13311; Ec, *Escherichia coli* ATCC 25922.

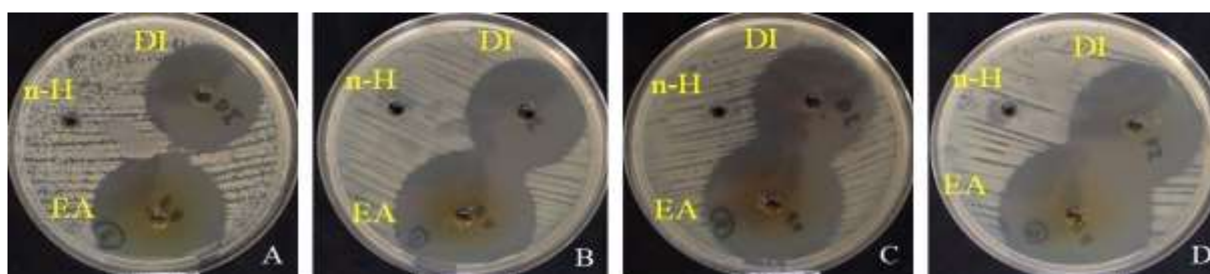


**Figure 4.** Antibacterial activity of ethyl acetate crude extract of *Penicillium griseofulvum* against pathogenic bacteria. **A.** *Bacillus cereus* ATCC 10876; **B.** *Citrobacter freundii* ATCC 8090; **C.** *Salmonella typhimurium* ATCC 13311; **D.** *Escherichia coli* ATCC 25922; **EA.** Ethyl acetate; **DI.** Dichloromethane; **n-H,** n-Hexane.

with maximal inhibition zone of 27.0 mm obtained against *C. freundii* ATCC 8090, followed by SDA and YMEA (no significant differences), with average inhibition zones of 15.4, 15.3 and 28.3 mm against *S. typhimurium* ATCC 13311, and 25.0 mm against *E. coli* ATCC 25922, respectively.

Data were presented as mean ± SD (n=2), means with the same letter are not significantly different ($p > 0.05$).

Anwar and Iqbal (2017) also studied the effect of growth media on antibacterial activity, and suggested that secondary metabolites are highly affected by the composition of the growth medium. Verma et al. (2017) found that starch was the source of carbon allowing the endophytic fungus to produce the highest amount of bioactive molecules followed by glucose. This is in agreement with the results obtained with MPR1 on PDA and MEA that contain the appropriate concentration of starch and glucose. The same results have been obtained with Mathan et al. (2013), where the PDA was the medium giving the best activity for *Aspergillus terreus.*

## Evaluation of antibacterial activity of crude extracts

Table 3 and Figure 4 show the antibacterial activity of different crude extracts of *P. griseofulvum* on agar well diffusion method. All the pathogenic bacteria were inhibited by ethyl acetate and dichloromethane crude extracts with the maximal activity showed against *E. coli* ATCC 25922 (45.5 and 41.0 mm respectively), unlike n-Hexane crude extract which did not inhibit any pathogenic bacteria.

The statistical analysis (Figure 5) showed that ethyl acetate was the best extraction solvent allowing the extraction of bioactive molecules (41.9 mm of average inhibition zones), followed by dichloromethane (35.7 mm of average inhibition zones). These can be explained that the bioactive molecules produced by this fungus are better extracted by polar solvents ethyl acetate and also dichloromethane than apolar like n-Hexane.

Data were presented as mean ± SD (n=2), means with the same letter are not significantly different ($p > 0.05$).

These results correspond to those obtained by Tong et al. (2014) where antimicrobial compounds of *Phomopsis* sp. ED2 and the endophytic fungus of medicinal herb *Orthosiphon stamineus* were mainly present in the ethyl acetate extract. Another study showed that the ethyl acetate extracts of two endophytic fungi isolated from *Ocimum citriodorum* Vis. show better activity than the other extracts (Mu'azzam et al., 2015).

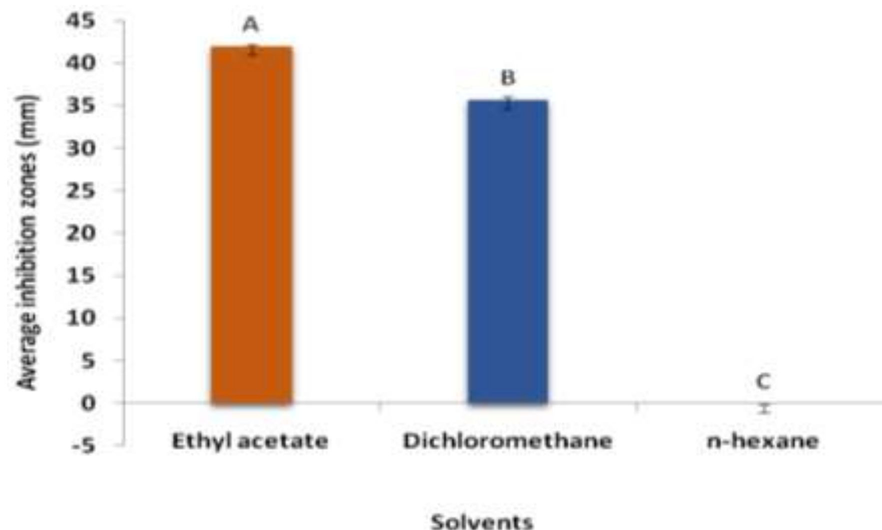


**Figure 5.** Effect of different extraction solvents on antibacterial activity of *Penicillium griseofulvum* against all pathogenic bacteria.

**Table 4.** MIC and MBC values (µg / ml) of endophytic fungus *Penicillium griseofulvum* against different pathogenic bacteria.

| Pathogenic bacteria | *Penicillium griseofulvum* | | Imipenem | Gentamycin | DMSO |
|---|---|---|---|---|---|
| | MIC | MBC | MIC | MIC | MIC |
| Bc | 100±0.0 | 100±0.0 | / | 0.25±0.0 | - |
| Sa | 100±0.0 | 100±0.0 | / | 0.50±0.0 | - |
| MRSA | 100±0.0 | 100±0.0 | / | 0.25±0.0 | - |
| Cf | 50±0.0 | 200±0.0 | 0.25±0.0 | / | - |
| St | 50±0.0 | 50±0.0 | 1.00±0.0 | / | - |
| Ec | 50±0.0 | 200±0.0 | 0.50±0.0 | / | - |

Bc, *Bacillus cereus* ATCC 10876; Sa, *Staphylococcus aureus* ATCC 25923; MRSA, Methiciline-resistant *Staphylococcus aureus* ATCC 43300; Cf, *Citrobacter freundii* ATCC 8090; St, *Salmonella typhimurium* ATCC 13311; Ec, *Escherichia coli* ATCC 25922; -, inactive.

## Determination of minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC)

MIC and MBC values (Table 4) were recorded for the crude ethyl acetate extract from *P. griseofulvum;* the MIC of this crude extract was of 50 µg / ml for the three Gram-negative pathogenic bacteria (*C. freundii* ATCC 8090, *S. typhimurium* ATCC 13311, *E. coli* ATCC 25922) and of 100 µg / ml for the three Gram-positive pathogenic bacteria (*B. cereus* ATCC 10876, *S. aureus* ATCC 25923, Methiciline-resistant *S. aureus* ATCC 43300).

According to Klepser et al. (1998), if the MBC/MIC ratio is ≤ 4, the extract is bactericidal and if > 4, it is bacteriostatic; and since MBC/MIC ratios obtained for all the test bacteria were ≤ 4 we can say that *P. griseofulvum* crude extract has a bactericidal effect against all pathogenic bacteria.

Broad spectrum antibacterial activity obtained by this endophytic fungus extract has already been observed with extracts of different endophytic fungi such as the endophytic fungi isolated from *Calotropis procera* (Rani et al., 2017).

Gram-negative bacteria are generally more resistant than Gram-positive bacteria and are therefore more difficult to contend, because their outer membrane protects the peptidoglycan cell wall. The results obtained in our study reveal the opposite, and this can be explained by the fact that the mode of action of the active molecules produced by *P. griseofulvum* MPR1 is not on the cell wall but possesses a different mode of action (Yenn et al., 2014). These results correspond to the results obtained with the extracts of other *Penicillium* such as *Penicillium* sp., an endophytic fungus of *Garcinia nobilis* that has been a source of new molecules with antibacterial activity mainly on Gram-negative bacteria (Jouda et al., 2014, 2016b). *Penicillium minioluteum* ED

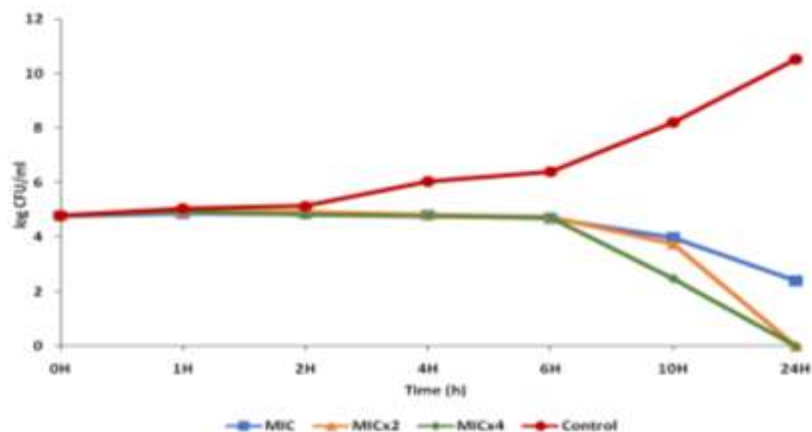


**Figure 6.** Time kill curve of *Penicillium griseofulvum* ethyl acetate crude extract against Methiciline-resistant *Staphylococcus aureus* ATCC 43300.

24 isolated from *Orthosiphon stamineus* Benth. also show better activity against Gram-negative compared to Gram-positive bacteria (Yenn et al., 2014).

## Time kill

The time-kill dynamic process is used for the evaluation of bactericidal or bacteriostatic effect of bioactive molecules; bactericidal activity is indicated by a 3-$\log_{10}$ reduction, the equivalent of 99.9% cell death, and the number of viable cells in the presence of the antibacterial compound (Balouiri et al., 2016).

Killing growth profile of *P. griseofulvum* ethyl acetate crude extract against Methicilline-resistant *S. aureus* ATCC 43300 shows a bactericidal effect at 24 h of incubation; 100% killing of the bacterial cells was achieved for MIC × 2 and MIC × 4 concentrations (Figure 6).

Previously, Ibrahim et al. (2015) reported the bactericidal effect of endophytic fungus *N. sphaerica* CL-OP 30 against Methicilline-resistant *S. aureus* at 24 h. The silver nanoparticules of *Penicillium polonicum* has also demonstrated bactericidal activity against a multidrug-resistant bacterium, *A. baumanii* (Neethu et al., 2018).

## Conclusion

In this study, we highlighted the antibacterial potency of endophytic fungi isolated from the medicinal plant, *M. pulegium* L. With preliminary screening we selected one isolate *P. griseofulvum* with a good antibacterial activity. This was confirmed after extraction, where ethyl acetate was the best solvent used for the extraction of bioactive secondary metabolites. The MIC/MBC and time kill of this extract show that it has a bactericidal effect against several bacteria. These promising results push us to want to determine the composition of this extract as well as its active fraction.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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# Health and nutritional quality assessment of *salmonella*-contaminated poultry products in sub-Saharan Africa; A case of cote d'ivoire

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**The poultry sector is developing rapidly in sub-Saharan Africa but it remains informal in many countries, including Côte d'Ivoire. This situation, which is favourable to the health and nutritional insecurity of food of avian origin, deserves special attention, because of *Salmonella*, which has poultry as its outbreak. The aim of this study was to help reduce the risk of food poisoning linked to the consumption of poultry products contaminated by *Salmonella*. A characterisation of avian production from the farm to the fork has been carried out. It covered 1860 samples of avian origin from 20 farms and 630 samples of diarrhoeal stool from human patients. The strains isolated were characterized by antibiotyping, serotyping and molecular typing. The frequency of isolation of *Salmonella* was 6.8% in poultry products, 5.8% in gizzards, 1.9% in carcasses, 8.2% in eggs and 4.2% in drinking water. In diarrhoeal patients, this frequency was 11.9%, with 15% in children and 10% in adults. Frequently isolated *Salmonella* strains were distributed among *S. Typhimurium* (27.6%), *S. Enteritidis* (20%) and *S. Hadar* (10.6%). A resistance of these microorganisms to β-lactamines was between 70% and 83%. Clonal links have been identified between the serotypes of *S. Typhimurium* and *S. Heidelberg*, isolated in both avian and human matrices. The study shows that unsanitary poultry products could be responsible for diarrhoeal *Salmonella* infections in humans; therefore, preventive provisions are needed for consumer health protection.**

**Key words:** Food insecurity, *Salmonella*, serotype, antibiotic resistance.

## INTRODUCTION

Diseases caused by microbial contamination of food are a major and growing public health problem. The situation is all the more worrying because despite awareness campaigns on food hygiene and good food preparation

practices advocated by sub-Saharan health authorities, the number of *Salmonella* isolated in laboratories remains significant (Dibi et al., 2017). Poor hygine practice in the industrial preparation of food and its storage outside the required standards facilitate the proliferation of microorganisms (WHO, 2005a). Most countries with a foodborne disease reporting system have demonstrated that for several decades there has been an increase in the incidence of diseases caused by microorganisms present in food, including the genus *Salmonella* (Jay et al., 2000). Worldwide, 90% of salmonellosis cases are foodborne (CNRSS, 2007); Africa leads with over 80% of cases (Newell et al., 2010). In Tunisia, this disease is still endemic, especially in rural areas, with an incidence of 5 per 100,000 inhabitants (WHO, 2005b).

Salmonellosis and particularly non-tyhoidal salmonellosis are responsible for sporadic or epidemic infections, most often due to food contamination or asymptomatic carrying (Rostagno and Callaway, 2012). Meat from poultry and pigs, eggs, dairy products, and green vegetables contaminated by manure or water are the most frequently cited vehicles or risk factors in the transmission of this bacterial agent (Filbert et al., 2012). The occurrence of salmonellosis is more frequent in persons at risk, in particular immuno compromised persons, including those infected by HIV/AIDS (Gordon, 2008).

In Côte d'Ivoire, studies show that *Salmonella* plays an important role in bacterial diarrhoea (Coulibaly et al., 2015). *Salmonella* promotes the occurrence of certain diseases such as meningitis (Sangaré et al., 2007) and various forms of rheumatism (Dakoury-Dogbo et al., 2001).

This study aimed to serotype *Salmonella* strains and determine their level of resistance to antibiotics generally used in Côte d'Ivoire.

## MATERIALS AND METHODS

The study included 2490 sample strains, including 1860 from poultry products and 630 from human diarrheal stools. The stools were received in a reference laboratory for the processing of human samples, with the consent of the participants. An average of 157.5 human samples were expected per year.

The non-biological material mainly included analytical equipment commonly used in bacteriology and molecular biology laboratories (culture media and reagents), as well as survey sheets used to assess the health characteristics and practices of poultry farms, poultry markets, slaughterhouses and their environment. The survey sheets also collected data on the type of meals consumed by some patients before the onset of infectious diarrhea (Table 1).

The isolation and identification of *Salmonella* strains from these samples was carried out in accordance with NF ISO 6579 (2002). Serotyping was carried out according to the scheme defined by Kaufmann and White, and then reviewed by Le Minor (Le Minor and Popoff, 1987 and 1997). The antibiotic susceptibility test was performed with standard antibiotics on all *Salmonella* spp strains (Kirby-Baeur method). The resistance study of the strains was determined by measuring the diameters of the inhibition zones, in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM, 2018).

## RESULTS

### Farm environment characteristics

The characteristics of the farm environment are divided into two groups of farms: those smaller than 3000 head (size 1) and those larger than 3000 head (size 2). Farms are generally characterized by the presence of rodents (rats, mice, etc.), insects and reptiles, also called pests (60%). These farms are generally located not far from the houses (55%). Among the farms studied, some were near traffic lanes (20%), landfill (15%) or slaughterhouses (10%). The farms in Adjamé commune are closer to the houses (20) than those in Cocody and Yopougon communes (Table 2).

### Characteristics of poultry markets

The study shows that all markets are open every day of the week. Where poultry sold is usually temporary (80%). These markets are characterized by facilities that do not meet health safety standards. The markets are also marked by the presence of rats, mice, insects and reptiles called pests (60%), and raving poultry (60%). These markets are often used as poultry breeding sites (60%). Two of the poultry markets visited are located near garbage dumps (Table 3).

### Characteristics of the last meals

The last meal taken by the people having made the diarrhoea gave the results contained in Table 4 which shows the diversity of the local menu composed mainly of attiéké, rice of alloko, foutou (banana, taro, yam, manioc), placali and kabato. This table shows that rice and fish are highly valued by the study population. Eggs come in second place, in terms of animal proteins.

### Identified serotypes

Five different serotypes were identified in this study. They

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**Table 1.** Distribution of avian origin samples.

| Years | 2006 | 2007 | 2008 | 2009 |
|---|---|---|---|---|
| Eggs | 125 | 125 | 125 | 125 |
| Carcasses | 120 | 120 | 120 | 120 |
| Gizzards | 120 | 120 | 120 | 120 |
| Water | 100 | 100 | 100 | 100 |

**Table 2.** Farm environmental characteristics.

| Indicator | Farms concerned per municipality N (%) | | | | | Total N (%) |
|---|---|---|---|---|---|---|
| | Abobo | Cocody | Port-Bouët | Yopougon | Adjamé | |
| Near dwelling | 3 (15) | 1 (5) | 2 (10) | 1 (5) | 4 (20) | 11 (55) |
| Watercourse | 0 (0) | 2 (10) | 2 (10) | 1 (5) | 0 (0) | 5 (25) |
| Pest access | 3 (15) | 2 (10) | 2 (10) | 2 (10) | 3 (15) | 12 (60) |
| Animals in divagation | 2 (10) | 2 (10) | 1 (5) | 2 (10) | 1 (5) | 8 (40) |
| Fully covered farmhouse | 3 (15) | 3 (15) | 2 (10) | 3 (15) | 3 (15) | 14 (70) |
| Proximity to high-traffic lanes | 1 (5) | 0 (0) | 0 (0) | 1 (5) | 2 (10) | 4 (20) |
| Near a landfill | 0 (0) | 2 (10) | 0 (0) | 0 (0) | 1 (5) | 3 (15) |
| Near slaughterhouse | 1 (5) | 0 (0) | 1 (5) | 0 (0) | 0 (0) | 2 (10) |

are: *S. Typhimurium*, *S. Enteritidis, S. Heidelberg, S. Dublin* and *S. Anatum*.

Table 4 shows the distribution of *Salmonella* strains from 2006 to 2009. Overall, it indicates a stationary evolution during these four years. However, in 2007, a significant number (25) were isolated. On average, 19 strains were isolated each year.

The distribution of these serotypes according to the 75 *Salmonella* strains isolated is shown in Table 5.

**Table 3.** Characteristics of poultry markets.

| Indicators | Number of markets (N) | (%) |
|---|---|---|
| Market opening 7/7 days | 5 | 100 |
| Pests | 3 | 60 |
| Temporary site | 4 | 80 |
| Divagating animals | 3 | 60 |
| Animal husbandry | 3 | 60 |
| Near dwelling | 2 | 40 |
| Bare floor | 2 | 40 |
| No changing rooms | 1 | 20 |
| Traffic nearby | 2 | 40 |
| Near a garbage dump | 2 | 40 |
| **Market cleaning | 0 | 0 |

The study focused on N markets, N= 5. The cleaning of cages and sales areas is done individually and once a week

### Level of resistance of *Salmonella* strains to common antibiotics

Table 6 expresses the frequency of resistance of the *Salmonella* strains studied; thus, despite the existence of a high number of strains resistant to families of antibiotics, there are still antibiotics whose strains showed no resistance.

Table 7 shows the frequency of resistance of identified strains to common antibiotics. Thus, one notes a strong resistance of the strains (72%) to ampicillins and amoxicillin + association. However, all *Salmonella* strains studied were susceptible to the following antibiotics: cefotaxime, imipenem, aztreonam, gentamicin, colistine. The resistance rates of *Salmonella* strains isolated according to the antibiotics tested are shown in Table 7.

## DISCUSSION

The farm investigation showed that undesirable animals such as mice, margouillats and rodents were accessible at these facilities. However, it is known that these animals may carry pathogens such as *Salmonella* (Corry et al., 2002). Cleaning farms is an effective way to reduce and even eliminate certain pathogenic microorganisms. However, in 30% of the farms, the soil consists of beaten earth, the cleaning of which does not guarantee the reduction of pathogens. Clay soils can provide favourable temperature and humidity conditions for the development of enterobacteriaceae, including *salmonella*. According to Cogan et al. (1999), concrete floors are easy to clean and disinfect and do not promote the survival and

**Table 4.** Survey of last meal taken per person before diarrhoea.

| Food | | Men | | Women | |
|---|---|---|---|---|---|
| | | Children (Nb) | Adults (Nb) | Children (Nb) | Adults (Nb) |
| Starchy | Attiéké | 35 | 115 | 57 | 199 |
| | Alloko | 20 | 10 | 43 | 72 |
| | Placali | 16 | 12 | 19 | 24 |
| | Foutou | 05 | 13 | 11 | 16 |
| | Kabato | 15 | 32 | 08 | 11 |
| | Rice | 45 | 196 | 30 | 241 |
| | Other | 06 | 03 | 05 | 08 |
| Animal protein | Fish | 85 | 203 | 44 | 192 |
| | Meat | 15 | 112 | 73 | 215 |
| | Egg | 38 | 57 | 49 | 153 |
| | Other | 04 | 09 | 07 | 11 |

NB / Child: between 4 and 17 years, adult: 18 years and over, other: no precise information on the food consumed before diarrhoea.

**Table 5.** Distribution of *Salmonella* isolates from 2006 to 2009.

| Serotype | 2006 (%) | 2007 (%) | 2008 (%) | 2009 (%) | Total (%) |
|---|---|---|---|---|---|
| *S. typhimurium* | 06 (08.0) | 11(14.7) | 07 (09.3) | 09 (12.0) | 33 (44.0) |
| *S. enteritidis* | 04 (05.3) | 08 (10.7) | 08 10.7) | 03 (04.0) | 23 (30.7) |
| *S. heidelberg* | 00 (00.0) | 04 (05.3) | 06 (08.0) | 02 (02.7) | 12 (16.0) |
| *S. dublin* | 03 (04.0) | 00 (00.0) | 00 (00.0) | 01 01.3) | 04 (05.3) |
| *S. anatun* | 00 (00.0) | 02 (02.7) | 01 (01.3) | 00 (00.0) | 03 (04.0) |
| Total (%) | 13 (17.3) | 25 (33.3) | 22 (29.3) | 15(20.0) | 75 (100.0) |

**Table 6.** Frequency of antibiotic resistance in *Salmonella* spp.

| Families | Antibiotic (concentration) | Resistance (%) |
|---|---|---|
| ß-lactamine | Ampicilin (10 ug) | 56 (74.7) |
| | Amoxicillin + Acide clavulanic (20 +10 ug) | 54 (72.1) |
| | Imipenem (10 ug) | 00 (0.0) |
| | Cefalotin (30 ug) | 52 (69.4) |
| | Cefotaxim (30 ug) | 00 (0.0) |
| | Aztreonam (30 ug) | 00 (0.0) |
| Aminosides | Kanamycin (30 ug) | 01 (01.6) |
| | Gentamicin (15 ug) | 00 (0.0) |
| Phenicols | Chloramphenicol (30 ug) | 38 (50.8) |
| Tétracyclines | Tetracycline (30 ug) | 30 (40.1) |
| Polypeptides | Colistine (50 ug) | 00 (0.0) |
| Nitrofuranes | Furans (300 ug) | 33 (44.1) |
| Quinolones | nalidixic Acid (30 ug) | 04 (05.6) |
| | Pefloxacin (5 ug) | 03 (04.2) |
| | Ciprofloxacin (5 ug) | 01 (01.6) |
| Sulfamides + combinations | Triméthoprim + Sulfamethoxazole (SXT) (1.25ug + 23.75 ug) | 45 (60.1) |

multiplication of bacteria. According to Huys et al. (2013), *Salmonella* strains are highly pathogenic bacteria and the presence of a single strain of *Salmonella* in a food product results in the food being declared unfit for

**Table 7.** Antibiotic resistance of *Salmonella* serotypes.

| Antibiotics | Resistant strains (%) | *S. Typhimurium (%)* | *S. Enteritidis (%)* | *S. Heidelberg (%)* | *S. Dublin (%)* | *S. Anatun (%)* |
|---|---|---|---|---|---|---|
| Ampiciline | 54 (72.0) | 27 (82.0) | 18 (78.3) | 05 (42.0) | 03 (75.0) | 01(33.3) |
| Amoxicillin+ Clavulanique Ac. | 54 (72.0) | 27 (82.0) | 18 (78.3) | 05 (42.0) | 03 (75.0) | 01(33.3) |
| Cefalotine | 52 (69.3) | 25(76.0) | 20 (87.0) | 04 (33.3) | 02 (50.0) | 01 (33.3) |
| Kanamycin | 01(01.3) | 01(03.0) | 00 (00.0) | 00 (00.0) | 00 (00.0) | 00 (00.0) |
| Chloramphenicol | 38 (50.7) | 26 (79.0) | 08 (34.8) | 02 (16.7) | 01 (25.0) | 01 (33.3) |
| Tetracycline | 30 (39.9) | 20 (61.0) | 07(30.4) | 02 (16.7) | 00 (00.0) | 01(33.3) |
| Nitrofurans | 33 (43.9) | 18 (54.5) | 11(47.8) | 03 (25.0) | 00 (00.0) | 00 (00.0) |
| nalidixic Acid | 04 (05.3) | 03 (09.1) | 01(04.3) | 00 (00.0) | 01 (25.0) | 00 (00.0) |
| Pefloxacin | 03 (04.0) | 02 (06.1) | 01(04.3) | 00 (00.0) | 00 (00.0) | 00 (00.0) |
| Ciprofloxacin | 01 (01.3) | 01 (03.0) | 01(04.3) | 00 (00.0) | 00 (00.0) | 00 (00.0) |
| Triméthroprime + Sulfamides (SXT) | 45 (59.8) | 30 (90.9) | 13(56.5) | 00 (00.0) | 00 (00.0) | 02 (66.7) |

consumption. Sanitary measures must therefore be taken to protect all products intended for human consumption). The relatively high rate of positive results of this work can be explained by several factors: non-compliance with standards, the absence of a general policy for monitoring farms, even private farms, the absence of *salmonella* surveillance and control programmes in hatcheries and private farms. The multiplication of carcass handling, the humidity and heat conditions offered in slaughterhouses, but also the slaughter and hygiene conditions throughout the slaughter chain allow the multiplication of *Salmonella* strains (Garnier, 2008).

Several other factors may be involved in *Salmonella* transmission; the persistence of infection in farm buildings and hatcheries certainly plays an important role (Gradel and Rattenborg, 2003). Rats and mice can carry infections and contaminate buildings and food (Van Immerseel et al., 2005).

Diarrhea has been observed in human patients after consumption of food of questionable hygienic quality. The dietary profile of their last meals, before the onset of diarrhoea, indicates overall that eggs are highly prized. However, it is recognized that eggs are an excellent reservoir of *Salmonella*, which is responsible for many diarrheal diseases in humans (De Knegt et al., 2015). Although in this study, it is difficult to say exactly which foods or other components of the meal or containers are responsible for infectious diarrhea. The positive *Salmonella* infection rate reported here is consistent with the ANSES Opinion (2012). This report showed that human cases of salmonellosis in *S. Enteritidis* were most often associated with the consumption of contaminated eggs and poultry meat, while cases of salmonellosis in *S. Typhimurium* are mainly associated with the consumption of contaminated pork, poultry and beef (Hugas et al., 2014). The analysis of *Salmonella* diarrhoeal stools isolated 75 strains of *Salmonella* grouped into five serotypes. The most frequent were *S. Typhimurium* (40.0%) and S. Enteritidis (30.7%). This result, which shows the prevalence of these two human *Salmonella* serotypes, is in line with the data from Grimont et al. (2007). In addition to *S. Typhimurium, S. Enteritidis*, *S. Heidelberg*, *S. Anatum* and *S. Dublin*, there are other serotypes such as S. Essen, S. Derby which are isolated from human biological products in Côte d'Ivoire, as shown by Coulibaly et al. (2010). According to a study conducted in Abidjan by the Microbiology Laboratory of the University of Cocody's Pharmaceutical and Biological Sciences UFR in 2001, the age group of people with typhoid fever out of 103 cases is 18 to 40 years old with a sex ratio of 0.9 (Mwamakamba et al., 2012). Tables 6 and 7 show respectively the families of antibiotics whose *Salmonella* strains are resistant and the resistance phenotypes observed in the isolated and identified strains. These results are all the more alarming as their resistance to β-lactamines, in particular ampicillin, amoxicillin + clavulanic acid and ticarcillin are important. Similarly, resistance of *Salmonella* isolates to tetracycline, Trimethroprime + sulfonamides and fluroquinolones was observed. This same observation is made by Ouédraogo et al. (2017), who questioned the main cause of this emergence of resistance. According to these authors, it is due to unsustainable consumption of antibiotics.

## Conclusion

The characterization of production systems in the study shows that the majority of selected poultry farms are deficient in terms of sanitation, protection of farm areas and farmer hygiene. The study showed sanitary deficiencies at poultry slaughter sites and poultry markets. Strains of *Salmonella* isolated both from poultry products and humans belong to different serotypes. The consumption survey showed several cases of *Salmonella*

diarrhoea following the consumption of certain food products. Although this prospective study cannot place direct responsibility for the food origin of *Salmonella* infectious diarrhoea in humans, it should be noted that diets combining poultry products have been identified as possible risk factors for *Salmonella* diarrhoea. The resistance to common antibiotics of *Salmonella* serotypes isolated is remarkable for some families of β-lactamines, particularly for Penicillins such as Ampicillin, Ticarcillin and Amoxicillin.

The uncontrolled use of antibiotic therapy in the treatment of certain diseases in humans and especially in agricultural practices could represent a real threat to public health.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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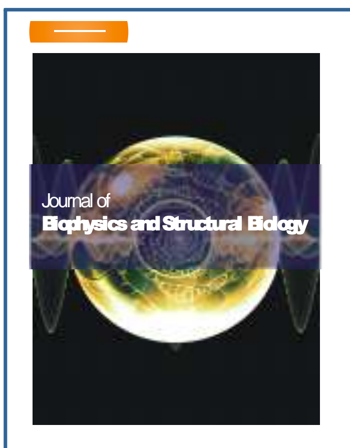

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