Heliyon 6 (2020) e05379

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Genotoxic impact of emerging contaminant amoxicillin residue on zebra fish (*Danio rerio*) embryos

Jayanta Chowdhury [a], Tapan Kumar Mandal [b], Sandhimita Mondal [a,*]

[a] Department of Microbiology, Techno India University, EM 4, Sector V, Salt Lake City, Kolkata, 700 091, West Bengal, India
[b] Department of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, 700 037, West Bengal, India

ARTICLE INFO

Keywords:
Amoxicillin
Residue
Zebra fish
Genotoxicity
HPLC
Food science
Agricultural science
Environmental science
Biological sciences
Cytotoxicity
Veterinary medicine
Health sciences
Toxicology

ABSTRACT

The widest-spectrum, most-consumed β-lactam antibiotic amoxicillin (AMX) is used to treat bovine mastitis that is caused primarily by many bacteria. Excessive use of antibiotics can lead to established residual contamination of the milk even after pasteurization. The amount of antibiotic residue above Maximum Residue Limit (MRL) has a negative impact on both public health and the environment. Therefore, the objective of this study is to determine the concentration of amoxicillin residue (AMXR) in raw and pasteurized milk samples of cow suffered from mastitis, by the standard methods of HPLC compared to pure AMX drug and effect of the said residue on the developmental toxicity and genotoxicity of zebra fish at 72 hpf and 48 hpf embryo, respectively. Results obtained by HPLC showed that AMXR exhibits 574.89 and 250.75 times higher concentration in the raw and pasteurized milk than MRL in compare to pure AMX drug. This current study showed that AMXR decreased the body length and yolk sac region, while the pure AMX drug-treated group showed increased height and length of the yolk sac and shorter body length relative to the other groups. The Comet Assay measured the DNA damage caused by AMXR. The group where AMXR were applied showed the highest percentage of tail DNA and tail moment relative to other groups. So, here AMXR is considered as the genotoxic contaminant that is emerging and affect on public health.

1. Introduction

Antibiotics are typically used for the treatment and prevention of the bacterial diseases even in animals. These have also been used in many domestic and food animals as a growth enhancer and mortality reducer by reducing the function of the immune system. These also minimize the nutrient wastage and the development of toxins (Graham et al., 2007) (Nisha, 2008). The antibiotic amoxicillin (AMX) is one of the most commonly used antibiotics in the world (Elizalde-Velázquez et al., 2016). It is an effective antibiotic agent against bovine mastitis which is primarily caused by bacteria (Saidi et al., 2019) (Ruegg, 2017). In India, mastitis causes major annual economic losses (Banerjee et al., 2017). Antibiotics are highly soluble in water and are the least usable biologically. Therefore, those residues were present in the marine environments (Zhou et al., 2018). AMX can be degraded by biotic and abiotic factors, but produces distinct intermediate products that are more resistant to breakdown and more toxic than the mother compound. These potent compounds can affect aquatic organisms in the aquatic environment and ultimately create an ecological imbalance (Elizalde-Velázquez et al., 2016).

Improper application of these antibiotics results the presence of antibiotics as a residual form in the milk. After excretion, a trace of the drugs might be found in milk, poultry, and egg (Sachi et al., 2019). Since 2006, antibiotic practice as a growth enhancer has been prohibited by European Union (EU) legislation (Cháfer-Pericás et al., 2010). Nisha (2008) reported that drug residues induce reproductive disorders, toxicity of the bone marrow, autoimmunity, nephropathy, hepatotoxicity affecting human health. It also reported that, the drug residue having carcinogenicity and mutagenicity properties (Nisha, 2008).

In recent decades, some chemical substances or compound have been identified by sophisticated methods of scientific detection technique which are characterized by a genuine or discerned threat to the environment or human health with a paucity of declared health criteria is called Emerging contaminants. Among such prevalent emerging contaminants include veterinary pharmaceuticals and drug residues (Lei et al., 2015). That residue has become a problem

* Corresponding author.
E-mail address: sandhimita@gmail.com (S. Mondal).

https://doi.org/10.1016/j.heliyon.2020.e05379
Received 29 May 2020; Received in revised form 28 July 2020; Accepted 27 October 2020

internationally (Teuber, 2001) in present day. This residue inhibits the microbes of the starter culture that produce fermented milk products such as yoghurt and cheeses (Seymour et al., 1988). Scientific literature revealed that, human beings are the ultimate users of these antibiotic residues (Nisha, 2008). It is reported that, pasteurization usually decreases the bacterial load present in milk, but will not reduce the amount of antibiotic residues (Ruegg, 2017). Furthermore, the proficient regulation of antibiotic residues in milk is crucial. Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) Codex Alimentarius Commission jointly laid down international standards for Food safety and monitoring efforts. Authorized Maximum Residue Limits (MRLs) for veterinary drugs in food are imposed in their products of treated animals with officially tolerable amounts of mother drugs or their metabolites that are harmless to consumers under National Council Regulation No. -EEC/2377/90 (Okocha et al., 2018). The maximum residue concentration is called the observable residue amount above the Maximum Residue Limit (MRL). These are created by the excessive use of a chemical in either the agricultural or veterinary sectors (Beyene, 2015). The embryo of zebra fish is a commonly used model for mechanism-based studies of developmental toxicity (Nishimura et al., 2016). Biomarkers in zebra fish have been reported to be altered by antibiotics (Oliveira et al., 2013). Zhang et al. reported that tetracycline generates oxidative stress and induces apoptosis that causes zebra fish embryos to disrupt development (Zhang et al., 2015). The genotoxic effects of agricultural pesticides on zebra fish were assessed by the comet assay (D'Costa et al., 2018). Thus, the objectives of this study are (i) to detect the presence of AMXR by using the HPLC method, in milk samples obtained at post therapeutic drug withdrawal period of 30-days from cows those have anamnesis of mastitis, and also quantification of AMXR of those samples compared to the standard AMX drug using the same technique; (ii) to detect AMXR in the same milk after pasteurization using the same method (iii) to investigate the effect of AMXR on the developmental stage of the embryo of zebra fish (iv) to assess the effect of AMXR as a genotoxic agent by Comet assay.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (ACTN) and other reagents used in this analysis were of analytical grade and obtained from SRL laboratories (India). Milli Q Elix (USA) used for water purification. The analytical grade of Amoxicillin (AMX) purchased from Sigma Aldrich (USA).

2.2. Collection of milk samples from cow

30 milk samples (100ml/sample) of about 10 herds in the dairy farming villages around the Kalyani, Nadia, West Bengal, India were collected from cows (i.e. 15 from clinical mastitis milk and 15 from subclinical mastitis milk) after examining the udder health of cows with a record of clinical and subclinical mastitis. The mastitis milk samples were confirmed by California Mastitis Test (CMT) and White Side Test (WST) (Mpatswenumugabo et al., 2017). The herds were marked on the day of first screening and after 30 days post screening anamnesis of the treatment regime was taken from the owners of that marked herds and those samples were collected and held in sterilised containers and stored at 4 °C for further use.

Collection of milk samples and experimental procedures reported in this manuscript followed the guidelines of the Committee for Control and Supervision of Experiments on Animals, (CPCSEA), India and procedures have been approved by the Institutional Animal Ethics Committee, WBUAFS, Kolkata [Ref. No. IAEC/67/xiv(B)] of 19.08.2019. In this research, animal experimentation was not performed. So, our present study did not sacrifice or anaesthetize any animals.

2.3. Amoxicillin residue (AMXR) screening from milk samples, its identification and quantification through High Performance Liquid Chromatography (HPLC)

All of these 30 milk samples were handled, processed and run in High Performance Liquid Chromatography (HPLC) Schimadzu Lc-20 AT system attached with Thermo ODS Hypersil C18 column (250 mm, 4.6 mm-ID; 5 μm particle size, USA) in accordance to the standard protocol for the finding of AMXR in milk samples (Schenck and Callery, 1998). For the analysis of amoxicillin residues in milk samples, along with optimization and validation of the analytical result, the simple and responsive HPLC protocol was followed (Ibrahim and Nasr, 2014). The detection was performed at a wavelength of 254 nm using a UV Vis SPD 20 detector. The mobile phase was prepared with acetonitrile and milipore distilled water after filtration and sonication. Examination of the results obtained by comparing the curve obtained from samples with that of the regular one. 20μl of processed milk samples were subjected to HPLC following standard protocol utilizing "LC Real Time Analysis" software. Then the findings obtained were analyzed by comparing curves obtained from samples with those of standard one. The AMXR quantification was performed by comparing the sample peak area to that of the standard AMX having the corresponding chromatogram (Kurjogi et al., 2019).

2.4. Treatments on zebra fish (Danio rerio) model

2.4.1. Determination of the maximum residue limit (MRL) of AMXR, obtained from milk samples by the process of HPLC and selection of drug residues for In vivo analysis

As a way to standardize the process, an aliquot of residue was obtained in acetonitrile and then we measured the Maximum Residue Limit (MRL) multiples in each sample. We deduced 1X MRL = 1 μl from the selected sample of drug residues for AMX. As this drug is frequently used against mastitis in the field of study, therefore AMX was taken into account. That's why we considered AMXR as a target contaminant in this study. The maximum residue concentration is the Maximum Residue Limit (MRL) that results from the registered use of an agricultural or veterinary chemical. It was recommended as appropriate on a food, agricultural product, or animal feed by legal and accepted authorization. The concentration is given in milligrams per kilogram of the commodity (or milligrams per liter in the case of a liquid commodity) (Boisseau, 1993).

Thus, we inoculated the drug residue obtained from a fraction of HPLC in 1 ml of E3 medium containing 10 nos. of just hatched 24 hpf zebra fish embryo at 25 well plate to monitoring their livability under 1x MRL inoculums for 24 h for amoxicillin residue (AMXR). Zebra fish eggs were maintained and hatched by following the modified method reported by Oliveira et al. (2013) (Oliveira et al., 2013).

We got 1066 X 1 MRL concentration of AMXR dissolved in acetonitrile in 1 ml of container obtained from one pasteurized milk sample, which we took for convenience of measurement, i.e. 1066 X 1 MRL in 1 ml i.e., 1000 μl. So, 1 μl = 1.066 X 1 MRL, i.e., 1 μl = 1 X MRL of amoxicillin residue (approx.) Here, 0.004ppm comprises 1 μl. Thus there was 4 μg in the 1ml or 1000 μl fraction collection vial. But the MRL of amoxicillin is 0.004ppm or 0.004 μg/ml.

We have got tolerance of absolute acetonitrile up to 14 μl by testing the livability of just hatched zebra fish embryos. As drug residues are dissolved in absolute acetonitrile so, in the 1X MRL amoxicillin residue containing sample, the concentration at 4 μg/ml of amoxicillin residue is dissolved in 1 μl, which was used for testing the livability of just hatched zebra fish embryos. It was noted that the maximium embryo was started dying at 3 X MRL of the pure drug (AMX) solution in acetonitrile. Therefore, the observed tolerance for AMX is up to 2 X MRL. Accordingly, just hatched and survived embryos of zebra fish in the E3 medium were charged with 2 X MRL AMX concentration of the same pure drugs in the E3 medium containing the same quantity of zebra fish embryo and 14 μl of acetonitrile were charged as well.

1μl of AMXR containing 0.004ppm residue was charged to the 1ml E3 medium. Thus, in our present analysis, 1ml or 1000 μl E3 medium contains 0.004μg/ml which is too harmful to zebra fish in the E3 medium. Untreated zebra fish embryos at E3 medium was acts as control.

But the concentration of AMXR in the extraction vial was 4ppm or 4μg/ml which is 1000 times more than the MRL value of AMX.

2.4.2. Impact of AMXR on zebra fish (Danio rerio) morphological traits

Treatments were administered on 48 hpf Zebra fish embryos and monitored after 24h development. Body length, yolk sac length and yolk sac height traits were included in the effects of AMX, AMXR and ACTN on the developmental process of embryos. Of each zebra fish, the body length was assessed at 72 hpf. This measurement was performed using an EVOS FL Auto (Invitrogen) fluorescent microscope. During this experiment temperature was held at 28 ± 0.5 °C (Zhang et al., 2015). The embryo analysis was performed using Image J 2.0 software.

2.4.3. Impact of AMXR on embryo cells of zebra fish by comet assay

A sensitive and rapid method for quantifying and analyzing DNA damage in individual cells is the Comet Assay, or single cell gel electrophoresis (SCGE). This assay has numerous applications, such as DNA damage, genotoxicity testing of new chemicals and pharmaceuticals, environmental biomonitoring etc. It also has the application for the assessment of genotoxicity and the efficacy of chemoprevention in cancer studies and testing also (Ostling and Johanson, 1984); (Singh et al., 1988).

Cell samples, including controls, were prepared to conduct this test by centrifuging trypsin-treated 24 hpf Zebra fish embryo cells exposed to 1X MRL amoxicillin residue (AMXR) i.e. 1 μl, 14 μl ACTN (acetonitrile) and 2 μl AMX (pure amoxicillin) for 24 h. Cells were centrifuged for 3 min at 7000 rpm, and then supernatant discarded. Then, the standard methods were used to treat and stain these cells (Frenzilli et al., 2004); (Olive and Durand, 2005) (Speit and Rothfuss, 2012). The photography of the slides was performed using an EVOS FL Auto (Invitrogen) fluorescent microscope.

These images of cells were marked one by one and analysis of comet study was done by Cometscore2.0.0.0 software.

The Tail DNA % and DNA migration (tail moment) are the parameter used for the DNA damage, which is the product of the length of the tail per the percentage of DNA in the tail (D'Costa et al., 2018) (Rocco et al., 2012). The sample numbers were enough for the statistical analysis of data to estimate genotoxic effects induced by the said treatment on the experimental model chosen (D'Costa et al., 2018) (Rocco et al., 2012).

The sample numbers on the selected experimental model is sufficient for statistical data analysis to estimate genotoxic effects caused by the procedure.

2.5. Statistical analysis

Graph Pad Prism 8 (Graph Pad Software, La Jolla, USA) was used for the statistical analysis of the experiments. The Student's-test was used for determination of AMXR. For developmental toxicity and genotoxicity, a one-way variance study (ANOVA, Kruskal-Wallis) based comparison rendered statistical significance between the untreated and treated groups.

3. Results

3.1. Detection of AMXR and determination of concentration by HPLC in milk samples

The trace of amoxicillin was primarily seen in the raw milk in all samples. In the case of pasteurized milk, 25 out of 30 samples showed traces of amoxicillin. So, in this study priority was given to the drug residue of amoxicillin (AMXR). Pasteurized milk samples revealed the existence of AMXR that was confirmed by the result of HPLC after comparison with the peak of the pure AMX drugs. In this current study, quantity of AMXR was measured to be 2299.56 μg/ml and 1003μg/ml in raw and pasteurized milk samples, respectively, (Figure 1). Therefore, the present study revealed that the quantity of AMXR in the raw and pasteurized milk respectively were 574.89 times and 250.75 higher than its MRL value, which is really alarming.

3.2. The effect of AMXR on zebra fish embryos

The finding of this present study regarding treatment of zebra fish after 24 hpf revealed that amoxicillin residue (AMXR) has adverse effects on the growth and development of the zebra fish. Compared to the untreated community (Figures 2a, 3a), the AMXR treated group shows reduced body length (Figures 2b, 2c, 2d, and 3a). The group treated with pure AMX shows an improved length of 1306.49 ± 12.04 μm of yolk sac and 494.647 ± 7.786 μm of yolk sac height and 2378.66 ± 13.80 μm of shorter body length (Figures 2c, 3b, 3c, 3a). The development of the embryos' body length was estimated at 72 hpf and the results are shown in Figures 2a, 2b, 2c, 2d and 3a and the length and height of the yolk sac are shown in Figure 3b and 3c. Compared with the controls, acetonitrile and amoxicillin have no major effect on physical appearance of the body. But the embryos had been healthy and active in the untreated community. The length of the body was 2613.86 ± 7.36 μm and could take up nutrients at 72 hpf from the yolk sac region. Zebra fish embryos exposed to ACTN and AMX exhibit 2461.09 ± 7.107μm, body length 2378.66 ± 13.80 μm. These display no major decreases in body length. But in the case of AMXR, the body length of 1729.1 ± 4.789μm shows that the increase in their delayed yolk sac absorption dramatically decreased their development. In the untreated category, no malformations were observed. Although, there was delayed absorption of yolk sac in the treated population. These findings showed that AMXR significantly decreases the growth of zebra fish embryos.

3.3. DNA damage measurement of zebra fish embryos by comet assay

The Comet Assay is a sensitive and fast technique used to measure and analyze individual DNA damage of the cell. The percentage of tail DNA and DNA migration (tail moment) are expressed here as a marker of the same.

3.3.1. Tail DNA%

Acetonitrile-treated cells show an improvement in tail DNA of 15.5 percent relative to the untreated community (Figures 4a, 4b, 5a). Treatment with AMX indicates 35.75 per cent tail DNA (Figures 4c, 5a). However, the highest 71.69 percent tail DNA compared to other groups

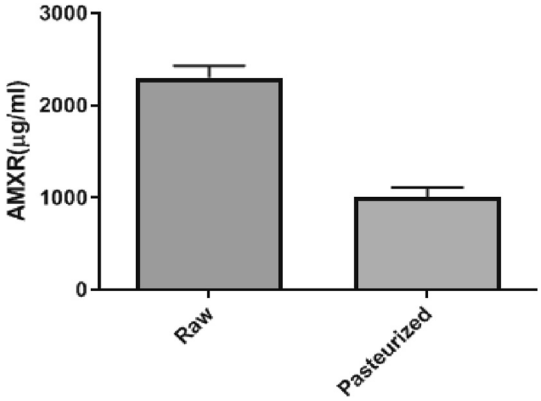

Figure 1. Concentration of Amoxicillin residue (AMXR) in raw and pasteurized milk determined by using high-performance liquid chromatography (HPLC). These experiments were repeated for three times with three sets. The data are shown as average values ±SD. $P<0.05$ defines significant difference of concentration between raw milk and pasteurized milk.

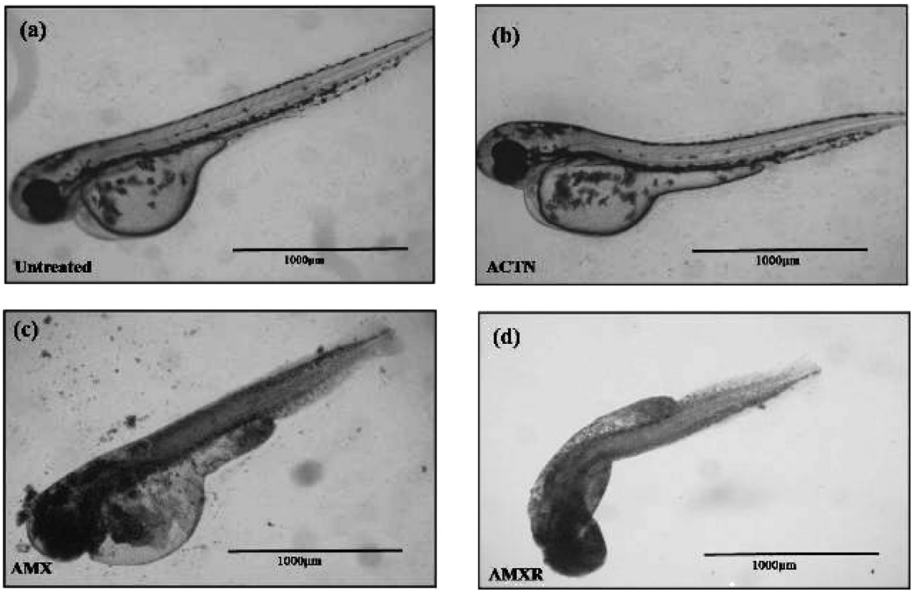

Figure 2. (a) Image of untreated *D. rerio* embryos at 72 hpf. (b)Image of *D. rerio* embryos malformations after ACTN treatment at 72 hpf (c) Image of *D. rerio* embryos malformations after AMX treatment at 72 hpf (d) Image of *D. rerio* embryos malformations after AMXR treatment at 72 hpf.

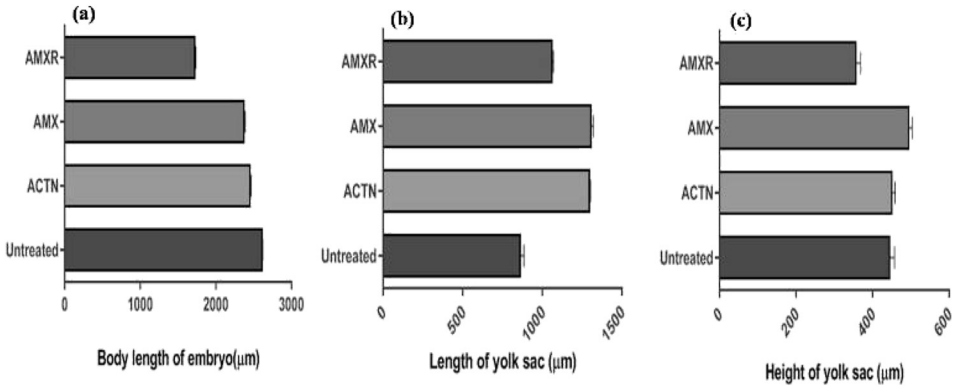

Figure 3. (a) Body length of *D. rerio* embryos without treatment (untreated) and with treatment of ACTN, AMX and AMXR at 72 hpf (b) Length of yolk sac of *D. rerio* embryos without treatment (untreated) and with treatment of ACTN, AMX and AMXR at 72 hpf.(c) Height of yolk sac of *D. rerio* embryos without treatment (untreated) and with treatment of ACTN, AMX and AMXR at 72 hpf. These experiments were repeated for three times with three sets. The data are shown as average values ± SD. P‘0.05 defines significant difference between untreated and treated cells.

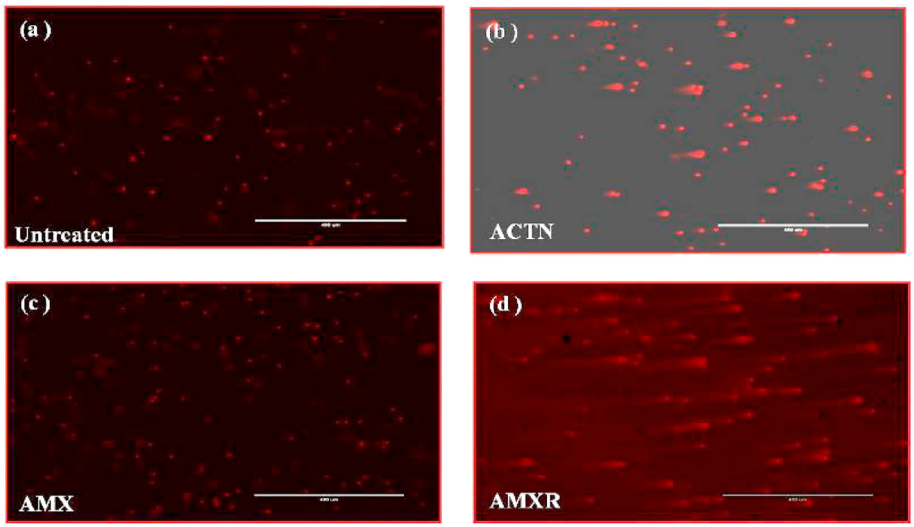

Figure 4. (a) Image of no DNA damage on untreated *D. rerio* embryos at 48 hpf. (b)Image of DNA damage of *D. rerio* embryos after ACTN treatment at 48 hpf (c) Image of DNA damage of *D. rerio* embryos after AMX treatment at 48 hpf (d) Image of DNA damage of *D. rerio* embryos after AMXR treatment at 48 hpf.

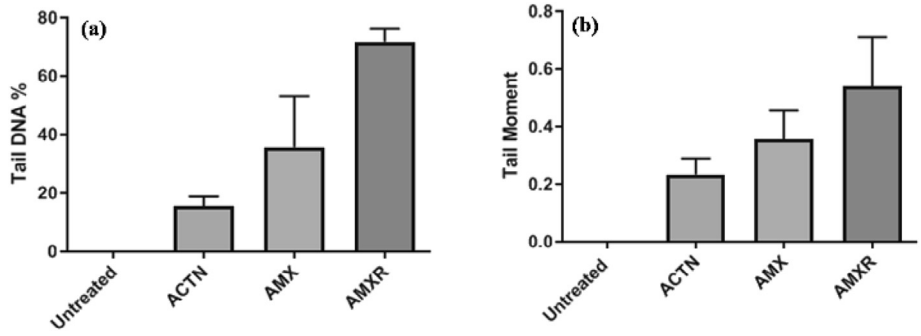

Figure 5. (a) Tail DNA (%) in cells of *D. rerio* without treatment (Untreated) and treatment with ACTN, AMX and AMXR at 48 hpf (b) Tail DNA moment in cells of *D. rerio* without treatment (Untreated) and treatment with ACTN, AMX and AMXR at 48hpf. These experiments were repeated for three times with three sets. The data are shown as average values ± SD. P˂0.05 defines significant difference between untreated and treated cells.

was observed in the AMXR treated community (Figures 4d, 5a). AMXR caused a rise in the percentage of tail DNA after just 24 h of treatment in this study. It is interestingly observed here that untreated cells and AMX show 35.75% tail DNA, while acetonitrile and AMXR treated cells show 15.51 and 71.69% tail DNA (Figures 4b, 4d and 5a,5b). In such cases, there was a difference in the percentage of tail DNA between AMX treated cells and untreated cells, which does not define a significant level of DNA damage. But AMXR-treated cells display the highest percentage of tail DNA under microscopy, which indicates substantial amount of DNA damage shown by comet tail.

3.3.2. Tail moment

In our analysis, we found a substantial increase in the tail moment of treatment with AMXR (0.53) relative to the untreated classes, ACTN (0.23) or AMX (0.35) (Figures 4a,4b,4c,4d, 5b).

4. Discussions

In veterinary medicine antibiotics are commonly used in the treatment of infectious diseases such as mastitis (Aalipour et al., 2015). Antibiotic residue monitoring is not used in standard quality control. Kurjogi stated that tetracycline residues at 70 °C and 100 °C had not been fully extracted from the milk and its products (Kurjogi et al., 2019). The MRL of amoxicillin in milk listed in Council Regulation No. 2377/90/EC by the European Commission is 4 μg/kg ('Forthcoming Papers,' 1990) (Sachi et al., 2019). Antibiotic residues that exceed the MRL in milk and milk products cause serious health problems for consumers (Van den Meersche et al., 2016). Antibiotic residues present in food materials can cause potential health effects, such as cancer and hypersensitivity reactions through bioaccumulation in muscle tissue, as well as the development of antibiotic resistance (Hassan, 2014).

We identified the AMXR here in raw and pasteurized milk. Milk pasteurization is the regular procedure for the milk packaging, paneer, curd, yoghurt, cheese production in the dairy industry. This AMXR reaches into this milk product and these items are consumed by the public with alarming concentration of the same. Here, the concentration of AMXR is high in raw milk and low in pasteurized milk, even 250 times that of pasteurized milk.

The pathway of amoxicillin degradation in aqueous medium was documented on 2013 by Gozlan et al. β-lactam containing a four-membered ring has been opened by hydrolysis and creates an intermediate product of AMX-penicillloic acid, which has an additional free group of carboxylic acids (Gozlan et al., 2013). pH has also a role in the degradation of AMX. In the alkaline scale, the lone pair electrons present on the amine group of the AMX-penicilloic acid is ready for nucleophilic attack on the carbonyl group. This yields a six member stable diketopiperazine ring which is the breakdown product (Elizalde-Velázquez et al., 2016).

Mastitis milk has an alkaline pH, which plays this role and can also generate this substance. Therefore raw milk possibly contains high AMXR.

Full removal of tetracycline has not occurred at high temperatures and degradation of tetracycline often depends on various factors, such as types of food matrix and cooking, etc (Kurjogi et al., 2019).

The factors behind the breakdown of the ß-lactam ring of amoxicillin are high temperatures and humid conditions (Naidoo et al., 2006). In our present study, this destruction of the ß-lactam ring structure is caused by pasteurization, so AMXR is present in the pasteurized milk at low concentration.

The vital link between environmental contamination and human health is fish. The harmful influence of antibiotic residues in the fish model, on the other hand, is not studied in depth (Zhou et al., 2018). We studied the effect of AMXR on the model of zebra fish. In our research, the adverse impact of AMXR is on the development of the zebra fish embryo. The length of the body is an significant marker of embryo development. The loss of nutrients may induce a shorter body length. In this AMXR study, AMXR reduces the body length of zebra fish and increased the length of yolk sac and decreases the height of yolk sac. During the early developmental stage, the yolk sac has a vital function, as it only provides food for embryos. The physical size of yolk sac will decrease along with the development of embryos (Zhang et al., 2015). AMXR demonstrates toxicity in development. The development of many enzyme activities such as alkaline phosphatase (AKP), acid phosphatase (ACP) and anti-oxidant response is also affected by antibiotics (Zhou et al., 2018). In fish muscle tissues, bioaccumulation of AMX takes place and results in immuno-allergic responses to consumer health (Elizalde-Velázquez et al., 2016). Here, AMXR may cause these reactions to induce developmental damage to the embryos.

We performed the Comet Assay to assess AMXR ′s role in the damage to DNA. The findings of our current analysis indicate a percentage increase in tail DNA and tail moment. Some studies have documented that erythromycin, lincomycin, ibuprofen, atorvastatin and gemfibrozil substantially confirm DNA damage after a short period of exposure and some antibiotics induced an increase in DNA migration (tail moment) and act as a genotoxic agent (Rocco et al., 2012). Toxins and chemicals cause Single-stranded DNA break. DNA breaks and DNA repair are more or less equilibrated in the *In vivo* setting. The quantity of DNA breaks increases if damage is greater than their repair capability. Diseases can evolve for this reason (Mozaffarieh et al., 2008). Comet assay proves its function with greater value and suitability as a bioindicator of genotoxic stress. In the analysis of this study, AMXR clearly demonstrated damage to the DNA and functions as genotoxic emerging contaminant.

5. Conclusion

The occurrence of antibiotic residue in the raw and pasteurized mastitis cow's milk by HPLC revealed that AMXR present 574.89 times

more its MRL level. But it is very surprising that, even after pasteurization, the presence of antibiotic residue is 250.75 times which is too alarming because in the Indian sense, the use of pasteurized milk is a common habit of the people before consuming milk. Yet literature is scanty that the drug residue is impacting the host system. Here, our experimental outcome showed that the bacteria were irradiated from the milk by pasteurization, but revealed that conventional pasteurization does not remove drug residues from the food chain. This affects the embryo of zebra fish in our research on *In vivo.* In our current research, AMXR influences body growth. AMXR influences the growth of body length and yolk sac region rather than AMX in our present research. Comet analysis found the highest 71.69 percent of tail DNA and the highest tail moment in AMXR, where 35.75 percent tail DNA was found in AMX. AMXR is the genotoxic material and an emerging contaminant. This is a pilot project, however, which involves a long-term prospective analysis to build up this view with more distinct antibiotics and more bovine herds spread in the first phase over other districts of West Bengal and then in the second phase over all Indian provinces.

Declarations

Author contribution statement

S. Mondal: Conceived and designed the experiments; Contributed reagents, materials; Analyzed and interpreted the data; Wrote the paper.

J. Chowdhury: Performed the experiments; Analysis tools and data; Wrote the paper with S.M.

T.K. Mandal: Contributed reagents, materials, analysis tools or data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors are grateful to the Chancellor, Techno India University, West Bengal for the provision of the requisite infrastructural and laboratory facilities. The authors are also thankful to Prof. Tarakdas Basu, Department of Biochemistry and Biophysics, University of Kalyani, Kalyani-741 235, West Bengal, India. for continuous support, infrastructure and laboratory facilities.

References

Aalipour, F., Mirlohi, M., Jalali, M., Azadbakht, L., 2015. Dietary exposure to tetracycline residues through milk consumption in Iran. J. Environ. Health Sci. Eng. 13, 80.

Banerjee, S., Batabyal, K., Joardar, S.N., Isore, D.P., Dey, S., Samanta, I., Samanta, T.K., Murmu, S., 2017. Detection and characterization of pathogenic Pseudomonas aeruginosa from bovine subclinical mastitis in West Bengal, India. Vet. World 10, 738–742.

Beyene, T., 2015. Veterinary drug residues in food-animal products: its risk factors and potential effects on public health. J. Vet. Sci. Technol. 7.

Boisseau, J., 1993. Basis for the evaluation of the microbiological risks due to veterinary drug residues in food. Vet. Microbiol. 35, 187–192.

Cháfer-Pericás, C., Maquieira, Á., Puchades, R., 2010. Fast screening methods to detect antibiotic residues in food samples. TrAC Trends Anal. Chem. 29, 1038–1049.

D'Costa, A.H., Shyama, S.K., Praveen Kumar, M.K., Fernandes, T.M., 2018. Induction of DNA damage in the peripheral blood of zebrafish (Danio rerio) by an agricultural organophosphate pesticide, monocrotophos. Int. Aquat. Res. 10, 243–251.

Elizalde-Velázquez, A., Gómez-Oliván, L.M., Galar-Martínez, M., Islas-Flores, H., Dublán-García, O., SanJuan-Reyes, N., 2016. Amoxicillin in the aquatic environment, its fate and environmental risk. In: Larramendy, M., Soloneski, S. (Eds.), Environmental Health Risk - Hazardous Factors to Living Species. InTech.

Forthcoming papers. Psychol. Sci. 1, 1990, 224, 224.

Frenzilli, G., Lenzi, P., Scarcelli, V., Fornai, F., Pellegrini, A., Soldani, P., Paparelli, A., Nigro, M., 2004. Effects of loud noise exposure on DNA integrity in rat adrenal gland. Environ. Health Perspect. 112, 1671–1672.

Gozlan, I., Rotstein, A., Avisar, D., 2013. Amoxicillin-degradation products formed under controlled environmental conditions: identification and determination in the aquatic environment. Chemosphere 91, 985–992.

Graham, J.P., Boland, J.J., Silbergeld, E., 2007. Growth promoting antibiotics in food animal production: an economic analysis. Publ. Health Rep. 122, 79–87.

Hassan, M.M., 2014. Antimicrobial resistance pattern against E. coli and Salmonella in layer poultry. Res. J. Vet. Pract. 2, 30–35.

Ibrahim, F.A., Nasr, J.J.M., 2014. Direct determination of ampicillin and amoxicillin residues in food samples after aqueous SDS extraction by micellar liquid chromatography with UV detection. Anal. Methods 6, 1523.

Kurjogi, M., Issa Mohammad, Y.H., Alghamdi, S., Abdelrahman, M., Satapute, P., Jogaiah, S., 2019. Detection and determination of stability of the antibiotic residues in cow's milk. PloS One 14, e0223475.

Lei, M., Zhang, L., Lei, J., Zong, L., Li, J., Wu, Z., Wang, Z., 2015. Overview of emerging contaminants and associated human health effects. BioMed Res. Int. 2015, 1–12.

Mozaffarieh, M., Schoetzau, A., Sauter, M., Grieshaber, M., Orgül, S., Golubnitschaja, O., Flammer, J., 2008. Comet assay analysis of single-stranded DNA breaks in circulating leukocytes of glaucoma patients. Mol. Vis. 14, 1584–1588.

Mpatswenumugabo, J.P., Bebora, L.C., Gitao, G.C., Mobegi, V.A., Iraguha, B., Kamana, O., Shumbusho, B., 2017. Prevalence of subclinical mastitis and distribution of pathogens in dairy farms of rubavu and nyabihu districts, Rwanda. J. Vet. Med. 2017, 1–8.

Naidoo, K., Nompuku, P., Mkalali, S., Shabangu, K., Nkabinde, L., Singh, V., 2006. Post-marketing stability surveillance: Amoxicillin. S. Afr. Fam. Pract. 48, 14–14d.

Nisha, A., 2008. Antibiotic residues - a global health hazard. Vet. World 2, 375.

Nishimura, Y., Inoue, A., Sasagawa, S., Koiwa, J., Kawaguchi, K., Kawase, R., Maruyama, T., Kim, S., Tanaka, T., 2016. Using zebrafish in systems toxicology for developmental toxicity testing: zebrafish and developmental toxicity. Congenital. Anom. 56, 18–27.

Okocha, R.C., Olatoye, I.O., Adedeji, O.B., 2018. Food safety impacts of antimicrobial use and their residues in aquaculture. Publ. Health Rev. 39, 21.

Olive, P.L., Durand, R.E., 2005. Heterogeneity in DNA damage using the comet assay. Cytom. Part J. Int. Soc. Anal. Cytol. 66, 1–8.

Oliveira, R., McDonough, S., Ladewig, J.C.L., Soares, A.M.V.M., Nogueira, A.J.A., Domingues, I., 2013. Effects of oxytetracycline and amoxicillin on development and biomarkers activities of zebrafish (Danio rerio). Environ. Toxicol. Pharmacol. 36, 903–912.

Ostling, O., Johanson, K.J., 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Biophys. Res. Commun. 123, 291–298.

Rocco, L., Peluso, C., Stingo, V., 2012. Micronucleus test and comet assay for the evaluation of zebrafish genomic damage induced by erythromycin and lincomycin. Environ. Toxicol. 27, 598–604.

Ruegg, P.L., 2017. A 100-Year Review: mastitis detection, management, and prevention. J. Dairy Sci. 100, 10381–10397.

Sachi, S., Ferdous, J., Sikder, M., Hussani, S., 2019. Antibiotic residues in milk: past, present, and future. J. Adv. Vet. Anim. Res. 6, 315.

Saidi, R., Mimoune, N., Baazizi, R., Benaissa, M., Khelef, D., Kaidi, R., 2019. Antibiotic susceptibility of Staphylococci isolated from bovine mastitis in Algeria. J. Adv. Vet. Anim. Res. 6, 231.

Schenck, F.J., Callery, P.S., 1998. Chromatographic methods of analysis of antibiotics in milk. J. Chromatogr. A 812, 99–109.

Seymour, E.H., Jones, G.M., McGilliard, M.L., 1988. Persistence of residues in milk following antibiotic treatment of dairy cattle. J. Dairy Sci. 71, 2292–2296.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.

Speit, G., Rothfuss, A., 2012. The comet assay: a sensitive genotoxicity test for the detection of DNA damage and repair. Methods Mol. Biol. Clifton NJ 920, 79–90.

Teuber, M., 2001. Veterinary use and antibiotic resistance. Curr. Opin. Microbiol. 4, 493–499.

Van den Meersche, T., Pamel, E.V., Poucke, C.V., Herman, L., Heyndrickx, M., Rasschaert, G., Daeseleire, E., 2016. Development, validation and application of an ultra high performance liquid chromatographic-tandem mass spectrometric method for the simultaneous detection and quantification of five different classes of veterinary antibiotics in swine manure. J. Chromatogr. A 1429, 248–257.

Zhang, Q., Cheng, J., Xin, Q., 2015. Effects of tetracycline on developmental toxicity and molecular responses in zebrafish (Danio rerio) embryos. Ecotoxicology 24, 707–719.

Zhou, L., Limbu, S.M., Shen, M., Zhai, W., Qiao, F., He, A., Du, Z.-Y., Zhang, M., 2018. Environmental concentrations of antibiotics impair zebrafish gut health. Environ. Pollut. 235, 245–254.